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The influence of eutrophication on methane production and its potential as a carbon source for zooplankton

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Keywords: methane flux; methane oxidizing bacteria; carbon isotopes; zooplankton grazing; food web

ABSTRACT

Methane constitutes a minor part in entire greenhouses emission, nevertheless its global warming potential is 20-30 times higher than carbon dioxide. Moreover, methane production and emission increases with eutrophication, and it has been demonstrated to serve as a carbon source for pelagic food webs. The methane oxidising bacteria (MOB) are the only group, which is able to utilize methane, as their sole source of carbon and energy and make a link between methane producers and higher food chain levels. However, the environmental controls of this alternative carbon source for plankton community in lakes with different trophic statuses are still poorly understood. Here we evaluated differences in fluxes of CH_4 and its potential as a carbon source for zooplankton in relation to the trophy state of two lakes. For this purpose two morphologically similar lakes with different trophy levels were investigated to determine methane efflux as well as zooplankton community structure and carbon stable isotope ratios in order to determine sources ($^{13}C/^{12}C$). The results revealed that C-CH_4 is incorporated into zooplankton through MOB community. Based on literature C-CH_4 has lower share of total carbon built into zooplankton in environments with higher trophy. However, our result showed that this process is more sophisticated and depends on food selectivity and vertical distribution of zooplankton members.

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1. INTRODUCTION

In lakes large quantities of organic matter are degraded under anaerobic conditions through methanogenesis, what contributes 30-80% of the anaerobic carbon mineralisation[1]. The produced methane volume strictly depends on the trophic state of the lake and increases in higher trophy[2]. A large proportion of the methane diffusing from the deep water layers can be utilized by methane-oxidizing bacteria (MOB)[3]. This is small but important group of bacteria that use CH_4 as their sole source of carbon and electrons. However, MOB need also oxygen as coreactant in the oxygenase reaction and as electron acceptor. Thus, their highest abundance occurres at the interface between anaerobic and oxygen zone. This bacterial biomass is available to zooplankton and protozoans. Although, MOB constitute ca. 3% of total bacteria, recent studies demonstrated that they may be an important component of zooplankton diet. Their significance increases with a limited amount of phytoplankton[4]. Further, the ability of zooplankton to exploit bacterial resources is not limited to the euphotic zones of lakes. Due to the vertical migration, seeking food in deeper water layers may be advantageous especially when zooplankton is forced to seek refuge from fish predation in deeper and dark water layers. However, the significance of methane-derived carbon to potential consumers under these conditions is largely unknown. Moreover, interactions in the food web, which influence on methane-carbon transfer are also little recognized. Thus, the aim of the study was to test hypothesis that C-CH_4 may consist a significant part in zooplankton food resources, and this is more depend on food selectivity and vertical distribution than on lake trophy.

2. METHOD

The study was carried out in two lakes with similar morphology, yet different trophy: Lagowskie Lake and Lodzko-Dymaczewskie Lake, located in Western Poland. Sampling and field measurements were conducted every month in the deepest place. Total phosphorus concentration was made according to standard methods[5]. Chlorophyll a concentration was determined by extraction with boiled ethanol, with a correction on pheophytin a. Aqueous concentrations of CO_2 and CH_4 were collected by headspace method and analyzed by gas chromatography. Water samples to MOB quantity analysis were taken from surface layer, aerobic-anaerobic interface and above bottom sediments. The MOB quantity was analyzed by real-time qPCR method. Zooplankton samples were collected from the same depth as MOB. 20 liters of water was filtered through 30µ, 100µ and 250µm mesh to separate the different size fractions. Then, individuals were selected and grouped under dissecting microscope. Values of isotopic carbon composition are expressed as conventional δ13C‰ notation (relative to international standard Pee-Dee Belemnite). The trophic conditions were estimated using the trophic state index (TSI) described by Carlson[6]. The classes of trophy were established as mean value of all TSI indexes (total phosphorus - TSI_{TP}, chlorophyll a concentration - TSI_{Chla}, Secchi disk visibility - TSI_{SD}).

3. RESULTS

The average TSI index value in Lodzko-Dymaczewskie Lake was 64, which confirmed the lake's strong eutrophy (Fig. 1). The $TSI_{TP} < TSI_{Chla} = TSI_{SD}$ relationship means that TP was limiting factor for algae development. However, chlorophyll a concentration at 55 µg dm^{-3} indicated strong phytoplankton bloom. TSI in Lagowskie lake (Fig. 2) indicate mesotrophy level (average TSI = 49). The opposite relation to Lodzko-Dymaczewskie Lake $TSI_{TP} > TSI_{Chla} = TSI_{SD}$ signalized that limiting factor for phytoplankton is other than TP, e.g. zooplankton pressure. At the same time chlorophyll concentration in lake was low (3 µg dm^{-3}).

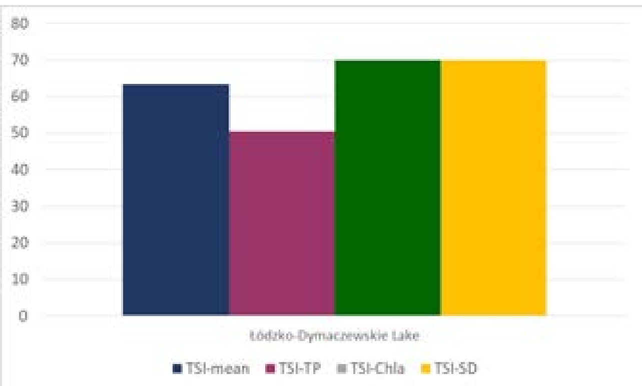

Fig. 1. Trophic state of Lodzko-Dymaczewskie Lake based on particular indexes

The ^{13}C signature of zooplankton was assessed throughout the summers 2016 and 2017 and the mean values for the crustacean community are given on Fig. 3.

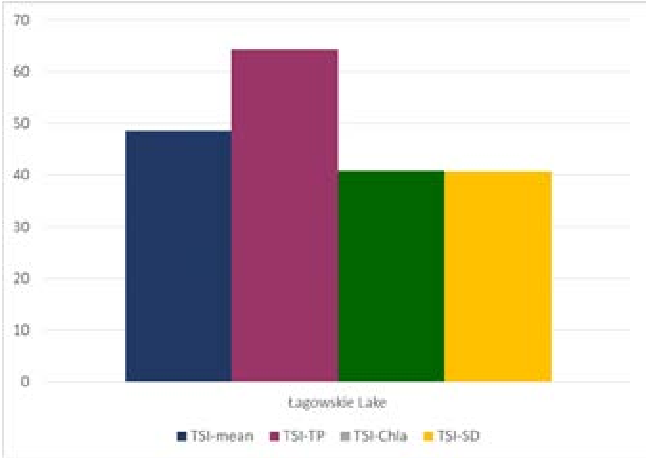

Fig. 2. Trophic state of Lagowske Lake based on particular indexes

The $\delta^{13}C$‰ were much lower in Lodzko-Dymaczewskie lake in comparison to lake Lagowskie. However there was a clear tendency towards lower values at deeper water layers. This pattern is correlated to the vertical methane concentrations (Fig. 4) which reaches highest values in hypolimnion of Lagowskie Lake and meta and hypolimnion of Lodzko-Dymaczewskie Lake.

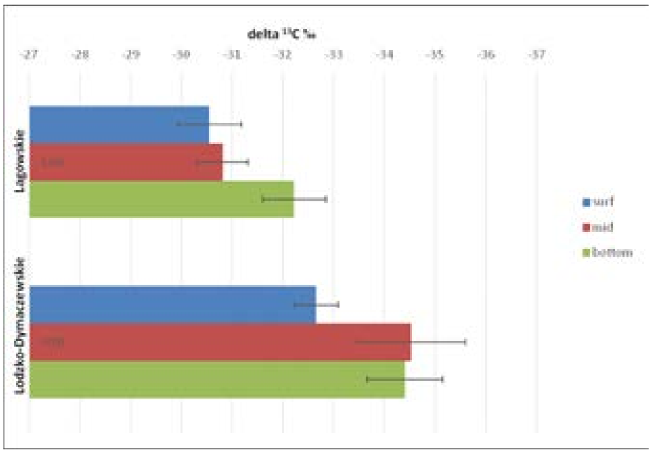

Fig. 3. The $\delta^{13}C$‰ signature of zooplankton on different level in Lagowskie and Lodzko-Dymaczewskie lakes

4. DISCUSSION

Transfer of carbon from CH_4 to higher trophic levels is mediated by methane oxidizing bacteria that are abundant at the water-sediments interface as well as in the suboxic hypolimnetic waters. The importance of this carbon source may depend on the prevailing biogenic gas production and emission pathway (diffusion or ebullition), on the activity and abundance of MOB, on the vertical distribution and grazing rates of zooplankton as well as their ability to exploit bacteria[7].

The importance of CH_4 for carbon budget in aquatic food chain strongly depended on the MOB abundance in the lake. The abundance and activity of MOB is regulated by the availability and concentrations of CH_4 and O_2 at the sediments interface and in the water column[8]. In consequence, the highest MOB concentrations were found in the middle level with oxycline and in the deepest water level.

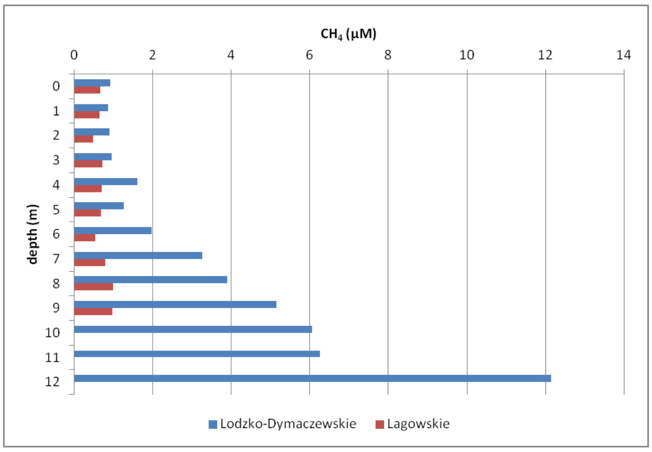

Fig. 4. Vertical profile of methane concentrations in the studied lakes

Methane oxidizing bacteria vary in size from 0.5 to 1.5 µm thus the importance of MOB food (and C-CH_4) for pelagic grazers may depend on their ability to efficiently exploit small sized particles. It was shown before that *Daphnia* sp. can efficiently graze on MOB community[9]. Therefore, larger zooplankton species, seeking refuge from predation in the dark hypolimnetic waters may gain additional energy feeding on more abundant MOB food.

5. CONCLUSION

The results revealed that C-CH_4 is incorporated into zooplankton body as result MOB consuming. However, the proportion of MOB in the zooplankton diet, expressed as lower values of δ^{13}C‰, is not always negatively correlated with the water trophy. These values are more related to vertical distribution of zooplankton. Deeper water layers in eutrophic lakes are characterized by a higher concentration of MOB, and simultaneously they play role as a shelter for zooplankton. Thus, zooplankton members staying at this layer may consume more MOB due to poor phytoplankton food base.

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O3-20

Long-term variation of CO_2 flux and the controlling factors in Asia's largest brackish water system, Chilika Lake

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Keywords: greenhouse gas, climate change, carbon dynamics, trophic status, source and sink of CO_2

ABSTRACT

Carbon dioxide (CO_2) is one of the greenhouse gas known to be the main driving factor of global warming; however, the role of the coastal ecosystem on global CO_2 fluxes remains unclear due to lack of adequate data. With an objective to bridge this knowledge gap, an estimation of CO_2 flux was made analyzing the 19 years data (1999-2018) from 30 sampling locations covering entire Chilika Lake, India. ANOVA test revealed a significant variation of CO_2 flux with respect to regions as well as seasons. The overall positive value of CO_2 flux indicates Chilika Lake acts as a source of CO_2 to the atmosphere. The northern region (NR) recorded highest release of CO_2, followed by the inlet (IR), central (CR) and southern region (SR). A significant relationship between apparent oxygen utilization (AOU) and excess DIC (dissolved inorganic carbon) in the NR indicates biological respiration is the major factor for rapid ventilation of CO_2 to the atmosphere. The sink of CO_2 and minimal C source in the SR could be attributed to the presence of seagrass bed and dominate primary productivity. The C estimation from this long-term data set (8.27×10^{-6} PgC Y^{-1}) indicates, Chilika contributes ~ 0.0019%C to global C emission to the atmosphere. The trend obtained through 19 years of monthly data indicated a significant annual increase of pCO_2 (μatm) and CO_2 flux (mmol $m^{-2}d^{-1}$) which are 1.57 & 0.048 respectively. At the same time, a significant decrease in pH @ 0.0006/year was observed which supports the phenomena.

1. INTRODUCTION

Nowadays global warming is a major concern. The increasing level of greenhouse gases such as CO_2, CH4, N2O, etc. are mostly responsible for the same. CO_2, being a major greenhouse gas and its impact on the biogeochemistry of coastal ecosystems, has drawn attention all over the world [1,2]. Lakes cover less than 2 % of the continent's surface but play a significant role in the global carbon cycle contributing significantly to C burial and emissions to the atmosphere. The lakes transform, store and exchange carbon with the atmosphere and considered frequently to be net heterotrophic as it promotes the emission of CO_2 to the atmosphere. Many studies have found that nearly 90% of the lakes are supersaturated with CO_2 with a net diffusion of CO_2 from surface waters[3]. Many pieces of evidence suggest that CO_2 supersaturation in lakes as the result of biological and abiotic in-lake processing of allochthonous organic matter.

Despite the importance of the above global issue, there is lack of studies on carbon biogeochemistry in Indian ecosystems except few (Hoogly estuary[4]; Godavari estuary[5]). Asia's largest brackish water lake and the first Ramsar site of India, Chilika Lake has been studied in this aspects only twice [2,6]. However, to have a better understanding of the ecosystem functioning of the lake, long-term time series data would be helpful. Hence in order to reveal the carbon dynamics, analysis of long-term time series data from (1999 to 2018) by Chilika Development Authority, Odisha, India was carried out. This study focused on following objectives: i) Estimation of CO_2 flux during last 19 years with respect to global C flux, ii) Understanding the controlling factors of CO_2 dynamics in Chilika Lake

2. METHOD

30 stations spread over the entire lake and the inlet channel were sampled on monthly basis during March 1999 to March 2018 (Fig.1). Sub-surface water samples were collected and *in situ* measurements were done for basic water quality parameters such as water temperature, pH, salinity, wind flow, depth and transparency. Dissolved oxygen (DO) measured following the modified Winkler's method. Total alkalinity (TA) measured using titration method. The pCO_2 was computed using measured salinity, temperature, nutrients (phosphate, silicate), pH and alkalinity using dissociation constants derived by Cai and Wang (1998) with a precision of 9-13 μatm[7]. Since Chilika maintains salinity range of 0 to 36 and the water temperature in the range of 20-35°C during the study period, Cai and Wang[7] was preferred over Millero[8] while computing pCO_2 using CO_2 SYS.XLS (version 14). CO_2 was calculated as a difference of pCO_2 of water and air [6]. The data for atmospheric pCO_2 for dry air for the period

of 1999 to 2012 obtained from WDCGG (World data centre for greenhouse gases) and the data of 2013 to 2018 from ESRL (GMD). The average was corrected for water vapour using the algorithms [6]. For seasonal comparison, all the 30 stations data from May, September and December (during 1999 to 2018) were considered as summer, monsoon and winter. In order to compare the data with respect to regions SR, CR, NR and IR, stations 8,17, 2 and 28 were considered.

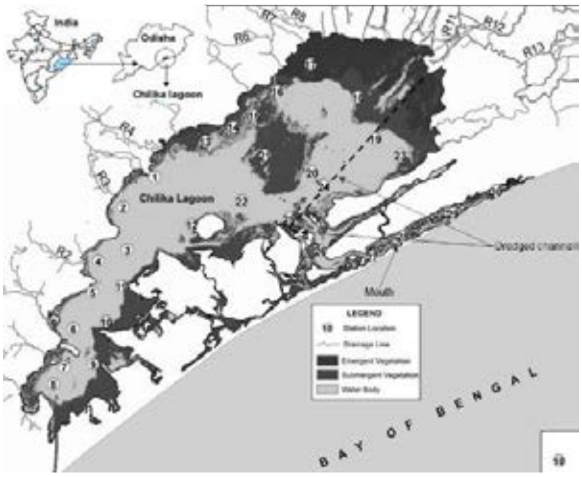

Fig. 1 Sampling map of Chilika Lake

3. RESULTS

The Chilika Lake recorded depth between 0.27 to 7.48 m with an average of 1.78 ± 0.93m didn't vary significantly throughout the study period. Higher depth mostly recorded in the IR followed by SR, CR and NR. The salinity ranged between 0 and 36 with an avg. 11.17 ± 9.20. Like other tropical coastal ecosystems, the salinity recorded minimum during monsoon due to the mixing of fresh water through riverine discharge and the level goes highest during peak summer when mixing of seawater through the Bay of Bengal becomes dominant. IR records the maximum salinity followed by SR (connected to the bay of Bengal through Rusikulya estuary), CR and NR (dominated with fresh water discharge from Mahanadi tributaries). There is no significant variation of salinity since last 19 years in the Chilika Lake.

The DO ranged between 0.3 and 14.69 with an avg.7.23 ± 1.67 mg lit^{-1}. Very low DO <4 only recorded at st-17 which could be due to decomposition of freshwater macrophytes. The average alkalinity of 113.98±34.37 mg lit^{-1} indicates the Lake maintains alkaline nature, however; it varies between 20.4 to 304 mg lit^{-1} according to the season and regions. The pH during the study period was ranged between 6 and 10.4 with an avg. of 8.2±0.53. The results of the $p$$CO_2$ and FCO_2 with respect to stations and seasons with a standard error bar has been shown in the Fig.2.

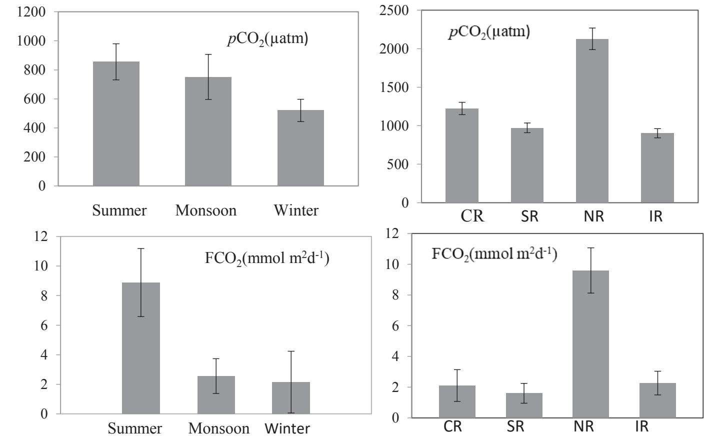

Fig. 2 Spatio-temporal variability of $p$$CO_2$ and FCO_2

4. DISCUSSION

The $p$$CO_2$ showed a pronounced gradient between the seasons. $p$$CO_2$ during summer was as high as 855.63 µatm, followed by monsoon (751.17 µatm) and winter (520.24 µatm). ANOVA test showed a very significant variation with respect to the season (n=1578, p=0.002). The observed values were quite lower than that observed in other Indian ecosystems such as in Mandovi–Zuari estuarine systems (monsoon: 2,250 µatm, non-monsoon: 2,600 µatm[9] but are near to the range as observed in the mangrove creeks of Godavari estuary (500– 6,500 µatm)[5]. The Chilika Lake also experienced significant variation in $p$$CO_2$ with respect to different regions (n=1578, p=0.002). The NR recorded the highest followed by CR, SR and IR.

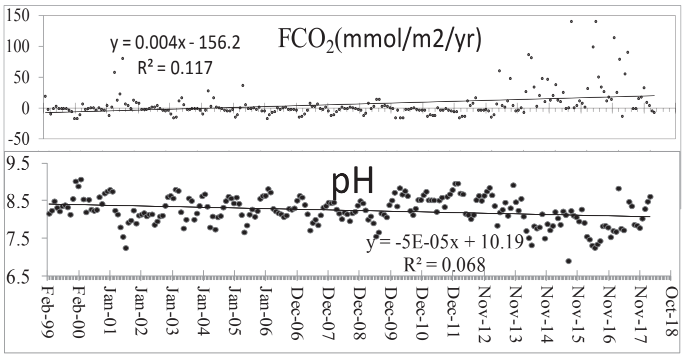

Fig. 3 Monthly variation of pH and FCO_2 in Chilika

The signature of summer, monsoon and winter are mostly found in May, September and December. Hence pulling together all the data of these months during the last 19 years, it was found that during summer and monsoon ~55% and in winter there was 71% occurrence of '–'ve CO_2 flux. This evidence that there are more chances of a sink of CO_2 during winter but rest of the seasons there is almost equal chances of source and sink of CO_2. Similarly, region wise data showed that the NR mostly results in the source of CO_2 (<40% '-'ve CO_2) and rest of the regions dominates sink of CO_2 (69%, 66% and 65% for CR, SR and IR respectively).

Out of 6870 values of calculated CO_2 flux (monthly data of 19 years from 30stations), 65% values were the negative indicating majority of the time the Chilika Lake behaves as a sink of CO_2. Rest 35% of data were positive indicates the source of CO_2 to the atmosphere. The mean of all the negative and positive values of CO_2 flux is -6.8 and 32.3 mmol m^{-2} day^{-1}. Although the occurrence of the sink was higher than that of the source, the quantity of CO_2 sink was relatively less than the CO_2 source. Hence, overall the Chilika behaves as a source of CO_2 to the atmosphere contributing to the global warming.

Station averaged (30 sampling stations) monthly data of last 19 years data showed a gradual significant increasing trend of pCO_2 and CO_2 flux with respect to time @0.1311 μ atm/month and 0.004/month respectively (Fig.3). pH showed an opposite trend (decreasing) @$5x10^{-5}$/month. This phenomenon could be the indication of acidification (though very minimal) and the impact of climate change (increasing C flux to the atmosphere) (Fig.3).

In order to compare with global carbon source and sink, the conversion of mmol $m^{-2}d^{-1}$ to $PgCyr^{-1}$ done considering the average surface area of $1000km^2$ (summer: 850 km^2 to monsoon:1150 km^2[2]). As per Richey et al. (2002)[10], the global C emission is 0.9 $PgCyr^{-1}$ and as per Borges (2005)[1], the carbon emission from global estuaries are 0.43 $PgCyr^{-1}$. The mean CO_2 flux from Chilika Lake during last 19years calculated as $8.27x10^{-6}$ $PgCyr^{-1}$ (1.888 mmol C $m^{-2}d^{-1}$) which is 0.0009% of the global carbon emission and 0.0019% of the emission from global estuaries (Chilika acts as an estuarine ecosystem as it is connected to the sea through a narrow mouth as well as a riverine system).

The net CO_2 efflux, in the NR (39.311 $x10^{-3}$ GgC d-1) was higher by 4 times than that observed in other regions (9.999 $x10^{-3}$ GgC d-1) of the Chilika Lake (Fig.2). A significant relationship between AOU and excess DIC in the NR indicates the biological respiration is the major factor for rapid ventilation of CO_2 to the atmosphere. The lowest flux in the SR could be attributed to lower bacterial respiration and higher primary production which utilizes the CO_2 [6]. Apart from this, most of the seagrasses in the Chilika Lake also covered in the SR and CR which could have also resulted in a significant sink of CO_2. Abnormally high pCO_2 (<10,000atm) and CO_2 flux recorded in the NR could be due to very low pH (<7) recorded in the NR due mixing of fresh water of low pH.

Excess DIC did not show any significant correlation with salinity indicates release of CO_2 not only confined to the freshwater region but throughout the salinity gradient recorded in Chilika Lake. Multi dimensional scale (MDS) showed two groups of the sampling stations. The first group includes the stations in the SR (most of the seagrass bed) and IR whereas, the rest of the stations from CR & NR (usually dominated with submerged macrophytes) formed the other group. This indicates the impact of submerged vegetation (macrophytes and seagrass) on the CO_2 flux in Chilika Lake.

5. CONCLUSION

The long-term monitoring of water quality is vital for understanding the ecosystem functioning. 19 years of monthly data revealed a significant rate of decrease of pH (0.0006/year) and an increase of CO_2 flux in the Chilika Lake which could be due to the impact of climate change on the ecosystem. The C estimation from this long-term data set indicates, in global perspective, the Chilika Lake contribute ~ 0.0019% C to global estuarine C emission to the atmosphere. Mineralisation of organic carbon, primary productivity and submerged vegetation are the controlling factors CO_2 flux in Chilika Lake. However, for accurate budget estimation, further study needs be focused on estimation of C flux with respect to seagrass bed, submerged, emerged and floating vegetation in Chilika Lake.

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O3-21

レジームシフト解析による霞ヶ浦での水質生態系変動要因の分析

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キーワード：時系列変動解析, レジームシフト解析, 気象パラメータ, 湖内水質パラメータ

抄録

環境分野で時系列変動解析は環境変動要因を分析する上で重要である。レジームシフト解析もその一つだが、水質や生物群集を対象としたレジームシフト解析により「水質・生態系がある安定な状態から他の安定な状態へ、安定状態の持続時間よりはるかに短い時間で遷移すること」の有無と遷移したタイミングとその内容を比較検討することができる。レジームシフトの起きるタイミングが各パラメータ間で同期していれば因果関係が強いと考える根拠となる。気象、河川水質、湖内水質に関する1970年代から2010年代までの連続環境パラメータの年平均値を用いてレジームシフト解析を行った。その結果、西浦では透明度が大きく低下した時期のはじまりと終わりに同調するように湖内の化学、生物、物理パラメータでもレジームシフトが生じていた。一方、北浦では、湖内水質パラメータで4回のレジームシフトが見られ、うち2回のシフトタイミングは流入河川の水質パラメータのそれとおおよそ一致していた。

1.　はじめに

近年、時系列変動解析に用いられる統計手法は深化し、多様になってきている[1], [2]。古くからある方法としてはフーリエ解析や移動平均を用いて、時系列変動を短期から長期までの個々の時間周期をもった時間変動成分の足しあわされたものとみなし、各時間変動成分を分離・抽出し、環境要因との関係性を議論されてきた[3]。また、一見して単調増加や単調減少している時系列データの場合でも、変動成分に分離せず、時間軸に対して単に線形回帰等を行い、増加、減少傾向を議論することは統計学的には支持されないことが明らかとなっている[4]。その理由としては、データ値が増加方向にも減少方向にもランダムに変動する場合でも、過去の値の変動を蓄積する時系列データでは、多くの場合、増加傾向か減少傾向のいずれかを示すことになるからである。このように、直感的に時系列変動を議論することのリスクを認識し、時系列変動解析にかけることで、統計学的根拠を担保しつつ変動要因の解析を進めることが本分野のグランドトゥルースになりつつある。

時系列変動解析は株価の変動に代表されるような経済指標の予測や環境分野では大気や海洋といった気候変動予測の分野で注目され、その解析手法も多岐にわたっている[1]。状態関数を用いて、観測誤差や観測時の異常性の影響を排除することで真の変動傾向を解析する方法や、ウェーブレットやSTARS等によるレジームシフト解析もその一つである[5]。レジームシフト解析はその名のとおりレジームがシフトしているか否かとその発生タイミングを検出する解析手法である。水質におけるレジームシフトを解析する場合、

「水質がある安定な状態から他の安定な状態へ、それぞれの安定状態の持続時間よりはるかに短い時間で遷移すること」

の有無と遷移したタイミングを解析することになる[6]。すべての水質でどの場所においてもレジームシフトが生じている保証はないことから、レジームシフト解析が、すべての水質変動機構の解明に資するか否かは現時点では不明である。しかしながら、長期間の時系列データであればあるほど、レジームシフトの起きている可能性は高く、そのタイミングが各パラメータ間で同期していれば因果関係が強いと考える根拠となる[5]。

湖沼環境の分野でレジームシフトの研究が脚光を浴びるきっかけとなったのはScheffer et al. (2001)による研究成果である[6]。この研究では、湖沼への栄養塩負荷に対する水草と植物プランクトンの現存量の変化をもとに、富栄養化すると突然植物プランクトンの優占する湖に変化すること、逆に水草の優占する湖沼に戻るには、植物プランクトンの優占するようなった栄養塩レベルよりも低い負荷量が必要であることを示した。この現象以外

にも、湖沼環境ではレジームシフトは下記の系で見られることをScheffer and van Nes (2004)は報告している[7]。1)貧酸素化による底泥からのリン回帰、2)浮葉植物の優占、3)湖底堆積物の安定性、4)ラン藻の優占。

石川ら(2006)は琵琶湖南湖で2000年以降急速に水草の優占するようになった点や港湾部でのハスやホテイアオイの群落の急激な消長はレジームシフトと考えられるとした[8]。霞ヶ浦においても、植物プランクトンの季節変化のパターンが 1993 年以前は夏季のラン藻と冬季の珪藻と緑藻の繰り返しパターンであったのに対し、1994年から2000年までは年間を通してラン藻が優占するようになり、2001 年以降は年間を通して珪藻がみられるパターンへと変化しており、レジームシフト様の変化を示している[9], [10]。

こうした事実から、日本の湖沼の生物・水質環境の変化の有無とその変動要因を解析する上で、レジームシフト解析は有効な分析手法の一つと考えられ、今回霞ヶ浦の長期モニタリングデータを対象に解析を行った。

2.　方法

レジームシフト解析に用いるデータソースは、以下の６つのカテゴリーにごとに分けて解析した。１）気象パラメータ群（気象庁および気象研究所の公開データ)、２）河川水質パラメータ群（国土交通省の公共用水域データ)、３）湖内物理パラメータ群（国土交通省の公共用水域データ)、４）湖内化学パラメータ群（国立環境研究所の霞ヶ浦モニタリングデータと国土交通省の公共用水域データ)、５）湖内生物パラメータ群（国立環境研究所の霞ヶ浦モニタリングデータ)、６）底質パラメータ群（国土交通省霞ヶ浦河川事務所のデータ)。

レジームシフトの同期性を議論する際に、より上位の環境因子で起こったレジームシフトが下位の環境因子に影響を及ぼす際の影響の強さによって、レジームシフトの発生の有無やそのタイミングが異なってくる可能性のあることも考慮した。湖沼の環境パラメータを物理と化学と生物の３種類のパラメータ群に分けた場合、水温や水深といった物理パラメータは化学パラメータや生物パラメータに大きな影響を与えるが、その逆は稀である。その意味で、湖内の物理パラメータ群は湖内の生物や化学パラメータより上位にくると言える（図１)。同様に、気象パラメータや流入河川の水質パラメータは湖内パラメータ群に大きな影響を与える一方、その逆は小さいことから、湖沼パラメータ群の上位に気象や流入河川水質のパラメータを配置した（図１)。

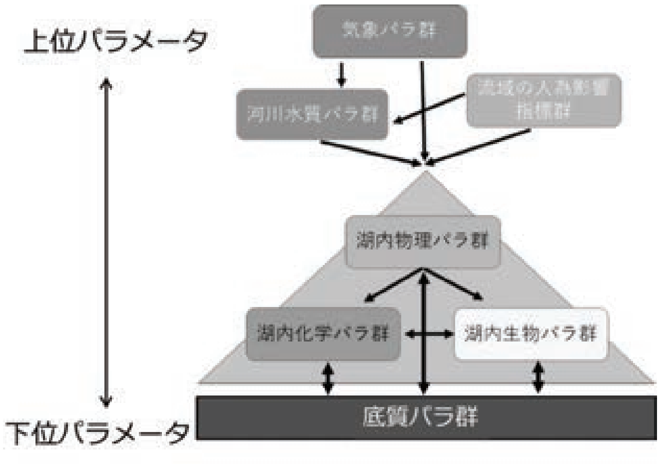

図 1 解析したパラメータ群の階層構造

レジームシフト解析はRによるパラメータ群ごとに主成分分析を行い、各主成分スコアの時系列データを用いて、Excel のマクロ機能（STARS）によりレジームシフトの有無とそのタイミングを決定した。５％の有意確率で、Cut-off Length は 10 年とし、Red noise estimation は IP4 を選択した。詳細はMöllmann and Diekmann (2011)に従った[2]。

もう一つのレジームシフト解析として、R ソフトウェア上で Chronological Clustering を読み出し、Vegan と rioja という R パッケージを読み込ませ、時系列クラスタリング解析を行った。本手法はDNA解析等でも多用されている通常のクラスタリング解析（似た配列を近い枝になるように配置する樹形図として出力される）の一種であるが、水質が似通った２つの年がある場合でも、それらの年が離れている場合には、樹形図で近い枝として配置されない等の工夫がなされている。

上記２種類のレジームシフトの解析結果を、霞ヶ浦の西浦と北浦別々に、水質に関連する上位パラメータ群から湖内パラメータ群まで、同一の時間軸上に整理しなおし、最長で過去 44 年間におけるレジームシフトの有無とその発生タイミングとその大きさ、内容に関する結果を比較検討した。

3.　結果

霞ヶ浦の西浦での上位パラメータ群から湖内パラメータ群までのレジームシフト解析結果を図2に示す。西浦と北浦でのレジームシフト解析により明らかとなった点は

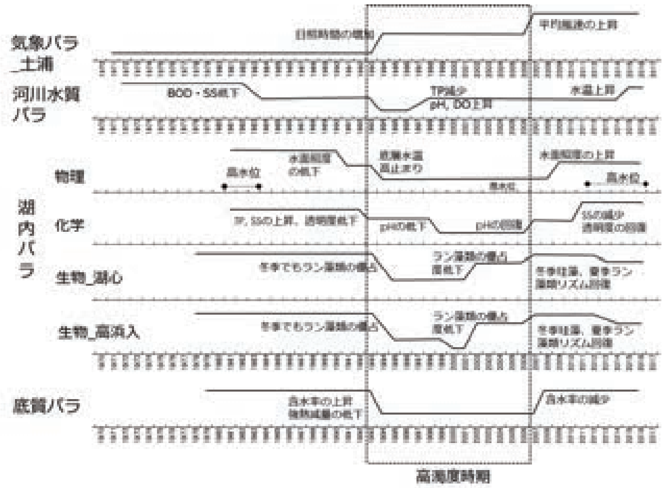

図 2 西浦での各種パラメータ群におけるレジームシフト発生状況の概略図

以下の4点である。

1）1993～1994 年を境に日照時間が増加した気象変化は、浚渫等による無機質な濁質成分の増加の時期と重なっていたが、ほぼ同時期に水質パラメータ群や生物パラメータ群においても大きなレジームシフトが起きていた（図2）。

2）西浦の湖内水質・底質と植物プランクトン相では高濁度時期（先述した無機質な濁質成分の増加時期）の開始時期、最盛期、終了時期とほぼ一致する 3 つのレジームシフトが検出された（図2）。

3）2010～2011 年を境に西浦では SS 濃度の減少や透明度の回復が見られており、夏季のアオコの発生や冬季の珪藻優占状態が観察されるようになった。

4）北浦では、1980 年代前半に流入河川水質の TP が低下した。湖内水質では 2003～2004 年を境に pH と底層 DO の上昇がみられ、2010～2011 年を境に透明度の回復が見られた。

4. 考察

得られたレジームシフトの結果をまとめた図2からも分かる通り、多くのレジームシフトは重大な環境要因の変化がその直前にある事が多く、またそうでないときには、どうしてシフトのタイミングが同期しなかったの原因を考えることができ、新たな発見や要因を見つけるきっかけにもなった。本レジームシフト解析結果で明らかとなった点は以下の3点である。

1）1993～1994 年を境に日照時間が増加した気象変化は、植物プランクトンの生産量や易分解性有機物濃度の増加を引き起こし、北浦では pH や DO の上昇につながった可能性が考えられた。一方、西浦では同時期に浚渫等による無機質な濁質成分の増加の影響が強く現れ、気象変化の影響を抽出することは難しかった。

2）西浦の高濁度時期は湖内水質・底質と植物プランクトン相に大きな影響を与えたと考えられ、その変化内容も濁質成分の増減で整合的に説明できるものであった。

3）日照時間が高止まりした 2000 年代後半には高濁度時期も脱し、西浦では SS 濃度の減少や透明度の回復が見られており、西浦では、アオコの発生をはじめ、新しい安定状態へとシフトしたと考えられた。

5. 結論

今回の霞ヶ浦のレジームシフト解析結果から、日本の湖沼の生物・水質環境の変化の有無とその変動要因を解析する上で、レジームシフト解析は有効であることが分かった。今後、気象変動影響や流域改変の影響評価をする上で有効な解析手法となりうる。しかしながら、ほぼ同時期に複数の外的要因に大きな変化が複数生じた際には、どの要因がより強く影響したかについて要因分離をおこなうことが困難な場合もあることが分かった。

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O3-22

池田湖の水質変動及び全層循環の発生要件について

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キーワード：全層循環, 水質管理, 気候変動

抄録

2018 年 2 月，6 年ぶりに池田湖の全層循環が確認された。そこで池田湖における水質変動について，DO 及び水温の調査結果を用いて解析を行った。DO の結果から，底層（200m）の調査が開始された 1983 年度以降，全層循環が確認されたのは，1984 年，1986 年，2011 年，2012 年及び 2018 年の 5 回であった。全層循環が確認されたとき，表層と底層の水温差は 0.2℃以下であり，また，全層循環の要件として，1 月及び 2 月の平均気温が関係し，より 1 月の平均気温の影響が大きいと考えられた。

1.　はじめに

図1に示す池田湖は，鹿児島県薩摩半島の南端に位置する湖面積 10.95km^2，最大水深 233m の九州最大の自然湖沼であり，約 6400 年前の火山活動に伴い形成されたカルデラ湖である。年間を通じて表層の水温が 4℃以上であることから，熱帯湖に分類される[1]。

池田湖の水質は，1955 年頃から周辺地域における社会活動の活発化に伴い，淡水赤潮が発生するなど水質悪化が顕在化した。このため，鹿児島県は，1983 年 3 月，池田湖の水質環境を保全することを目的に，「池田湖水質環境管理計画[2]」を策定し，総合的な水質保全対策を推進している。

その結果，表層における池田湖の水質は概ね良好な状態を維持し，OECD の栄養レベルに基づく調和型湖沼の分類では，中栄養から貧栄養に分類されるようになった。

図1　池田湖位置

一方，池田湖においては，循環が湖底まで到達しない状態が 1987 年から 2011 年までの 25 年間，継続したことに伴い，無酸素状態の継続や底泥からの窒素，りんの溶出など，底層部における水質悪化が顕在化していた[2]。

このような中，2011 年に全層循環が確認され，その後，2012 年，2018 年と従来よりも短いスパンで全層循環が確認されたことから，本研究では，池田湖の水質変動及び全層循環の発生要件について，DO，水温及び気象データを用いて解析を行ったので報告する。

2.　方法

調査は，最大水深地点に設定されている基準点 2 において，年 6 回（偶数月）実施した。

調査期間は，底層（200m 層）の調査が開始された 1983 年 4 月から 2018 年 2 月までとした。

調査項目は，DO 及び水温を設定した。

DO は，バンドーン採水器で採水し，試験室にて JIS K 0102 により分析を行った。

水温は，メモリー水深水温計（アレック電子株式会社（現:JFE アドバンテック株式会社）製 ABT-1）を用い，表層から底層までの水温を現地で記録した。

気象データについては，鹿児島県指宿の気象観測所のデータ[3]を使用した。

3.　結果

3.1　DO

図 2 に表層（0.5m層）及び底層における DO の経年変化を示す。底層の DO が表層と概ね一致したときを全層循環とした場合，1983 年以降において全層循環が確認されたのは，1984 年，1986 年，2011 年，2012 年及び 2018 年の 5 回であった。

層別では，表層の DO は，概ね，春季から夏季にか

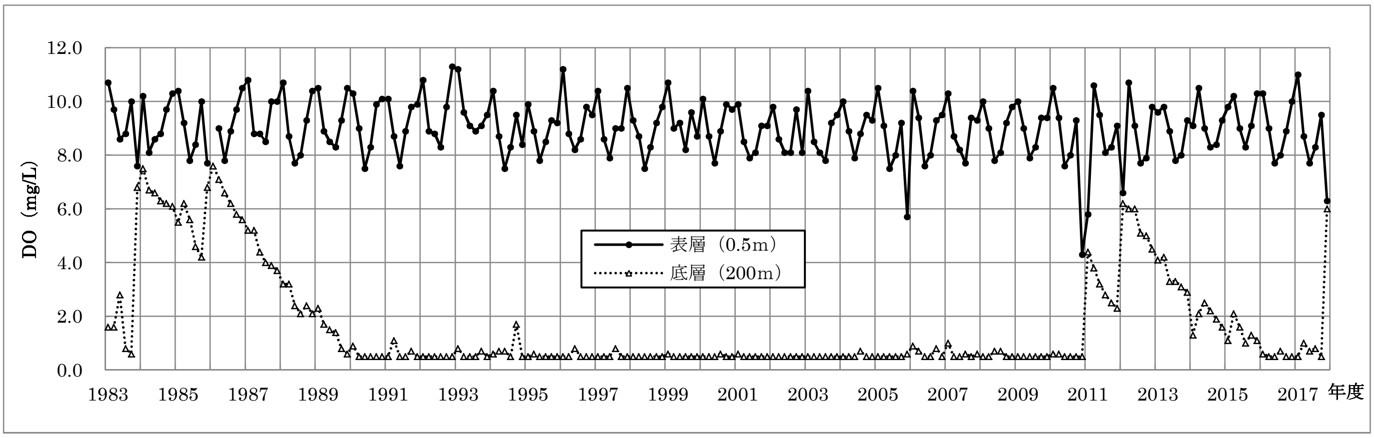

図 2　表層及び底層の DO の経年変化

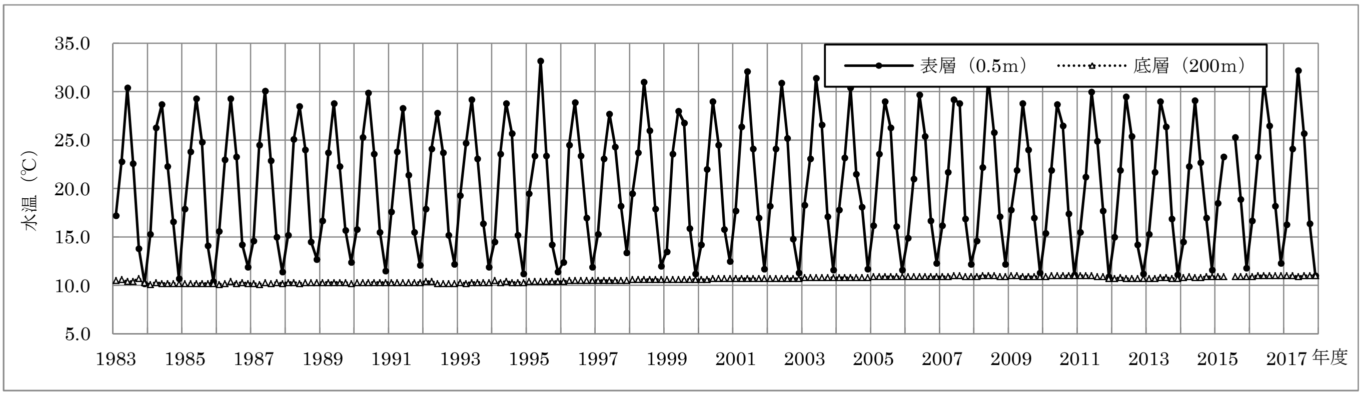

図 3　表層及び底層の水温の経年変化

けて減少し，秋季から冬季にかけて増加するといった季節変動が確認された。しかしながら，全層循環が確認された年においては，底層との混合による影響と考えられ表層 DO の低下が冬季に確認された。底層は全層循環発生時に DO が上昇し，その後低下するといった明瞭な変動が確認された。なお，1986 年及び 2012 年の全層循環をみると，全層循環後に新たな全層循環が発生しない場合，概ね 4 年程度でほぼ無酸素状態となっていた。

3.2　水温

図 3 に 2 月の表層及び底層の水温の経年変化を示す。表層の水温は，春季から夏季にかけて上昇し，秋季から冬季にかけて低下するといった季節変動を繰り返していた。

一方，底層の水温については，1984 年 4 月が 10.1℃と最も低く，その後上昇傾向を示し，2018 年 2 月は 11.0 と最も高く，その上昇率は約 0.027℃/年であった。これは，鹿児島における 1931～2016 年の気温変化率[4]（2.8℃/100 年＝0.028℃/年）と概ね一致していた。

2 月における表層及び底層の水温について図 4 に示す。全層循環が確認された年は，表層と底層の水温差が 0.2℃以下のときであった。

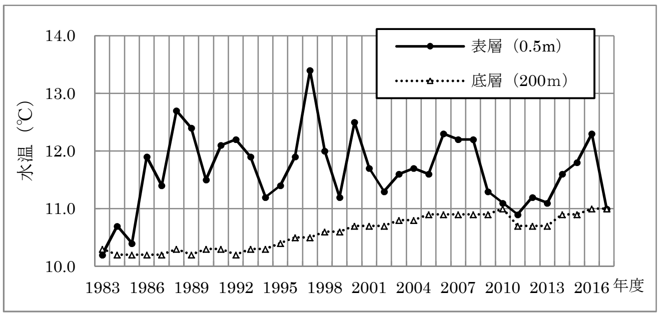

図 4　2 月における表層及び底層の水温の経年変化

4.　考察

全層循環の発生には，主に次の 2 つの物理過程が考えられる[5]。

(1) 気温の低下と冷たい季節風の吹き出しによって冷却された湖面水が，強制対流によって下方にもぐりこむ。重くなった表面の水が徐々に沈み，下方の水と混合しながら下降する。

(2) 湖岸の水が冷やされて，密度流となって湖底に流れ込む。

池田湖においては，流入河川は延長 2km 未満の小規模な河川のみであることから，河川からの冷たい水により湖岸の水が冷却されることは考えにくい。

したがって，池田湖における全層循環は，前述(1)のとおり，気温と風速が関係していると考えられる。

ここでは，気温の気象データを使用し，池田湖における全層循環の発生要件について，検討を行った。

指宿観測所における 12 月から 2 月までの気温の推移を図 5 に示す。1 月の平均気温が 7℃を下回る場合，又は 1 月の平均気温が 8℃以下でかつ 2 月の平均気温が 9.5℃を下回る場合のいずれかの要件で，全層循環が確認された。

よって，池田湖における全層循環の要件として，1 月及び 2 月の平均気温が関係しており，より 1 月の平均気温の影響が大きいと考えられた。

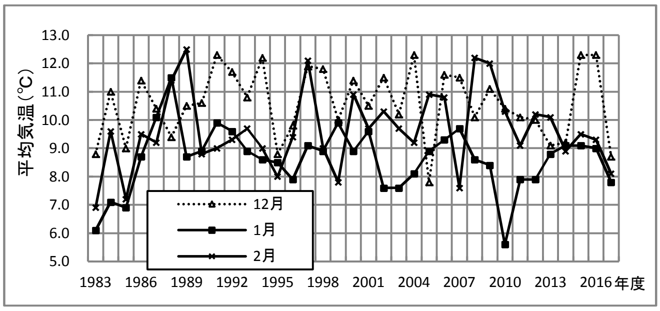

図 5　指宿観測所における平均気温の推移

5. 結論

全層循環前後における池田湖の水質変動に及び全層循環の発生要件について，DO，気温，水温の結果を用いて解析を行ったところ，以下のことが明らかになった。

(1) 1983 年以降において全層循環が確認されたのは，1984 年，1986 年，2011 年，2012 年，2018 年の 5 回であった。

(2) 底層の水温は上昇傾向を示し，上昇率は約 0.027℃/年であった。これは，鹿児島における 1931 ～2016 年の気温変化率（2.8℃/100 年＝0.028℃/年）と概ね一致していた。

(3) 2 月の調査結果から，表層と底層の水温差が 0.2℃以下のときに全層循環が確認された。

(4) 池田湖における全層循環の要件として，1 月及び 2 月の平均気温が関係しており，より1月の平均気温の影響が大きいと考えられた。

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O3-23

Study on similarity of COD fluctuation in Lake Kasumigaura and Lake Teganuma

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Keywords: Water pollution, Water quality management, Moving average, COD

ABSTRACT

Lake Kasumigaura, Teganuma and Inbanuma are lakes located in an area with the same meteorological conditions,total catchment areas are with a radius about 30 km^2. Lake Kasumigaura is a symbol of Tsuchiura City, the second largest lake in Japan. On the other hand, Lake Teganuma and Inbanuma are located in Chiba Prefecture and are used as a place for people's relaxation. Moreover, it is used as drinking water in Lake Inbanuma and important water resource for people. Although they are independent lakes, and external factors are influenced to fluctuations COD, but the annual values of COD are similar to over the years. Therefore, in this study, we analyzed COD fluctuation for Lake Kasumigaura and Teganuma that have 1 hour COD data. As a result, clear variations in COD fluctuations are found, indicating that the COD is fluctuating due to external factors. It is necessary to consider the fluctuation of COD value due to external factors in analyzing the water quality influenced by dredging and purification water guidance conducted in the lake.

1. INTRODUCTION

After the war, wastewater from factories and households were discharging into public water in Japan. As a result, the water quality of public water were dirty and caused people's health problem. In 1986, when a water quality standard were enacted as a water pollution prevention law, 11 lakes in Japan are chosen as water quality natural lakes. Field observation, and numerical analysis, investigation and research have been conducted for many years in order to find out comprehensive and systematic water quality conservation measures in these lakes. However, environmental standard such as the Chemical Oxygen Demand(COD) and Biochemical Oxygen Demand(BOD) of river used as an organic pollution indicator in public water are lower than that of sea and river[1]. **Figure 1** shows the location map of Lake Teganuma, Kasumigaura and Inbanuma (hereinafter referred to as 3 lakes) which are the objects of this study. The catchment area of 3 lakes is within a radius of 30 km and is located in an area with similar weather condition. **Table 1** shows parameters of 3 lakes. The average depth of Lake Kasumigaura is 4.0 m deep and about 4 times deeper than Teganuma, the catchment area is about 17 times larger than the Teganuma and Inbanuma. 3 lakes pollution has become a problem since the late 1900s, and we have been working on water quality improvement for many years. In Lake Teganuma, the COD value was high during 27 consecutive years from 1974 to 2001 and it was the worst in Japan. By the operation of the Kita-Chiba headrace tunnel, the water quality were improved about half of the 1990's. However, it still showed high COD value compared with others. **Figure 2** shows the change over the years of COD in 3 lakes. The figure is important at the beginning of this research. The change over the years of COD are similar for 20 years until the operation of Kita-Chiba headrace tunnel is started in April 2000. In general, COD fluctuation factors can be divided

Table 1 Parameters of Teganuma･Kasumigaura･Inbanuma

	Area [km^2]	Lake volume [km^3]	Average depth (Max depth) [m]	Basin area [km^2]	Basin population [people]
Kasumigaura	172.0	850 million	4.0(7.0)	2156.7	970,000
Teganuma	6.5	190 million	0.8(3.8)	143.9	510,000
Inbanuma	11.5	50 million	1.7(2.5)	493.9	780,000

Figure 1 The location map of Lake Teganuma･Kasumigaura ･Inbanuma

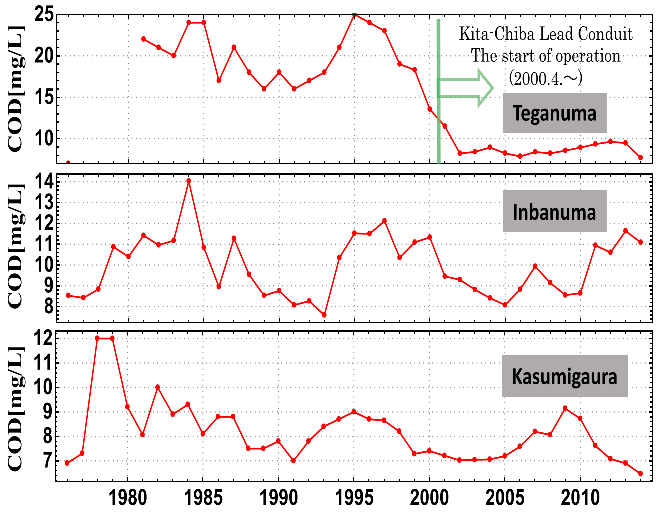

Figure 2 The change over the years of COD in 3 Lakes(Inbanuma:Jyousuidousyusuigutisita , Teganuma:Teganumachuo, Kasumigaura:mean of Kosin · Asouoki · Kakeumaoki · Tamatukurioki)

into internal factors that organic matter is generated by plankton in the lakes and external factors due to pollutant loads discharged from outside the lakes. From the above, 3 lakes are an independent lake, it is considered that external factors have influenced the COD fluctuation. So far, many studies aimed at elucidating water quality variation characteristics for each lake have been done, but in order to grasp water quality variation due to external factors for multiple lakes have not yet. In this study, we will concentrate on external factors such as weather, focusing on the relationship between rainfall and water quality variation.

2. RESULTS

2.1 COD fluctuation after rain

The water quality date of Lake Kasumigaura and Teganuma (hereinafter referred to as 2 lakes) was observed at a water quality automatic observation station (Teganuma:Teganumachuo, Kasumigaura:Kosin) managed by the Ministry of Land infrastructure and Transport. And, the rainfall of 2 lakes were observed at ground rain gauge (Teganuma:Abiko, Kasumigaura:mean of Tutiura · Tukuba · Simotuma · Kakioka · Minori) managed by the Japan Meteorological Agency. 2 lakes COD fluctuation were observed within 3 days after observing rainfall intensity 10 mm/h. At this study is defined that increase or decrease of COD is increased or decreased by 3 mg/L or more from monthly average COD value in each year. The reason for choosing 3 mg/L or more is that the daily variation of COD in the two lakes is mostly distributed from 1 mg/L to 3 mg/L. The reason for setting it to 10 mm/h or more was set with reference to the value of 10 mm/h to 20 mm/h, which is defined as a slightly strong rain in the Japan Meteorological Agency. As a result, there were 77 cases of rain events that recorded rainfall intensity of 10 mm/h or more during 1997 to 2011 in Lake Kasumigaura, 15 cases which the COD increased after rainfall, and 1 case was decreased. The other hand, in Lake Teganuma, there were 88 cases of rain events that recorded rainfall intensity of 10 mm/h or more during 2002 to 2014, 17 cases in which the COD increased after rainfall, and 5 cases was decreased.

2.2 The similarity of daily average COD

Changing of average COD value is illustrated for the purpose of checking the trend of COD fluctuation. **Figure 3** shows 15 day moving average (7 days before and after) of daily average COD in 2 Lakes. In this study moving average of 7, 15, 60, 180, 360 days is showed. In this paper, we describe the 15-day moving average that is the shortest number of days even though similarities are found in the fluctuation of moving average in 2 Lakes. The similarities of COD fluctuations in 2 Lakes are seen from 2004 to 2009 and, The peak of COD value

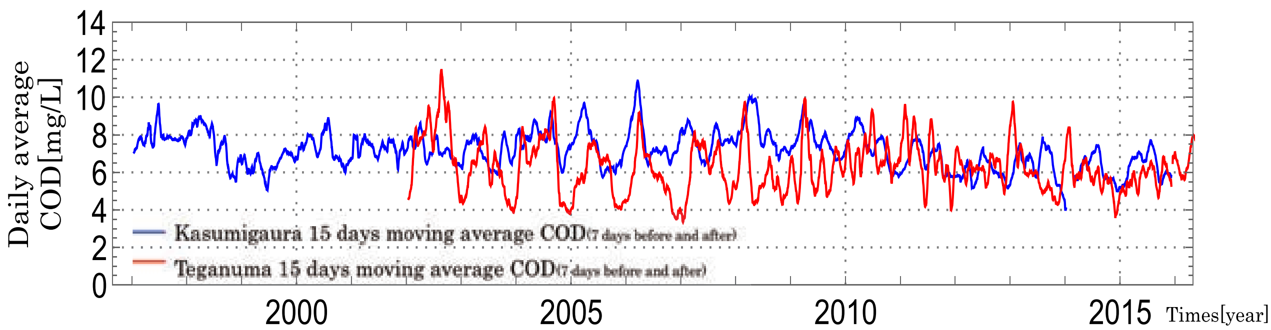

Figure 3 15 day moving average of daily average COD (7 days before and after)

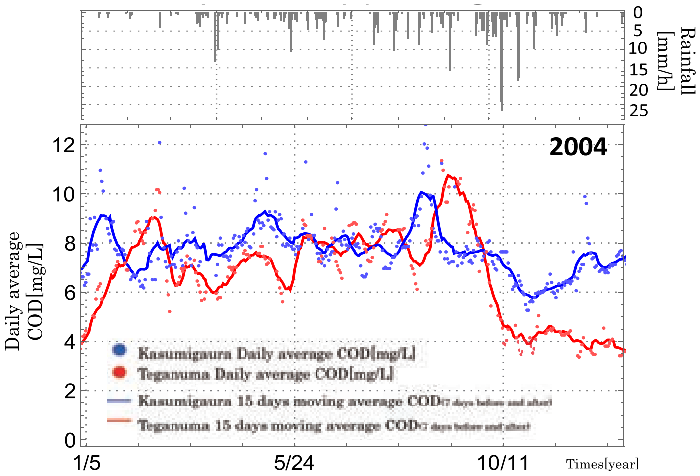

Figure 4 15 day moving average (7 days before and after) of daily average COD in 2 Lakes and rainfall (Lake Kasumigaura: Tutiura)

in Kasumigaura can be seen at an earlier time in Teganuma. **Figure 4** shows the 15 day moving average of daily average COD value when the similarity of the COD fluctuation was most characteristically observed in 2 Lakes and rainfall at Lake Kasumigaura. The peak of COD value in Teganuma can be seen about 1 month after the peak of daily average COD in Lake Kasumigaura.

3. Consideration

3.1 Regarding COD increase/decrease after rain

There are two reasons why COD increases after rain with rainfall intensity of 10 mm/h or more. The first is that the COD increases as COD of outside water is higher than the COD of inside the lake flows the lake. The second is that plankton, which is an internal factor of COD increase, increases COD by ingesting and propagating substances flowing from the outside of the lake. On the other hand, there are two possible reasons why COD decreases after rain. The first is that the COD decreases as the lake water is diluted. Secondly, the COD is reduced by the influent from the outside of the lake extruding the water staying inside the lake to the downstream. About 20 % of all cases in 2 Lakes COD increases after rain with rainfall intensity of 10 mm/h or more. On the other hand, the decreases in COD are less than 10 % of all cases. In contrast to showing that COD increases after rain with a rainfall intensity of 10 mm/h or more, as a reason for not showing a decrease in COD, the amount of water diluted or spilled out to the lake was shortage.

3.2 The similarity of COD fluctuations

The fact that the peak of the moving average of daily average COD value in 2 Lakes was observed at almost the same time can be thought to be an increase in COD due to external factors. From this it can be said that it is necessary to consider the COD fluctuation due to external factors caused by climate change in improving water quality such as dredging and vegetation purification in the lake.

4. Conclusion

(1)The COD increased by 3 mg/L or more within 3 days after rain with rainfall intensity of 10 mm/h or more in the 2 Lakes. On the other hand, it was shown that COD does not decrease more than 3 mg/L within 3 days after rainfall.

(2)The peak of the 15 day moving average of the daily average COD in 2 Lakes is seen almost at the same time and the peak of Lake Teganuma can be seen about 1month after the peak of Lake Kasumigaura.

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O3-24

琵琶湖における有機物指標の検討

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キーワード：水質保全, 有機物管理, COD_{Mn}, TOC

抄録

環境基準としての COD は, 水質汚濁の程度を示す指標とされ, 汚濁防止に一定の役割を果たしてきたが, 現代では COD の指標を見直すべきである。本研究では, 琵琶湖における定期調査より COD と TOC のデータの比較を行った。その結果, 琵琶湖水の COD 値は有機物の汚濁レベルを見る上では差し支えないが, 有機物の絶対量を量る指標としては問題があった。水質汚濁対策が進み, 流域からの有機汚濁負荷が削減された結果, 湖内の内部生産が有機物の主体となっている。これからの対策には, 外来魚の増加, 水草の大量繁茂, 在来魚類や二枚貝等の著しい減少等, 生態系の脆弱化の問題と合わせ, 上記問題の解明には生態系の物質収支の把握を進める必要がある。

1. はじめに

日本国の水質汚濁防止の枠組みは, 公共用水域の水質汚濁にかかる環境基準を設け, 行政目標として基準を満たすよう事業所等からの排水規制や下水処理等の対策が講じられてきた。環境基準としての BOD, COD は, 利水条件により階級化されており, 水質汚濁の程度を示す指標として, 汚濁防止に一定の役割を果たしてきた。BOD, COD は微生物学的視点や測定の簡便さなどの利点があり有効な指標であるが, 一方で有機物の絶対量を把握できないことや, 河川は BOD, 湖沼や海域は COD とする基準の不統一について昔から議論があった。近年は, 水域の汚濁削減対策が進み, 著しい水質汚濁が減少してきたにもかかわらず, 全国の多くの湖沼では COD 環境基準値を達成できていないことから, COD の指標を見直す動きがある。また, 公共用水域の水利用の変遷や多様化によって, 水環境の保全には水質汚濁を防止するだけでなく, 豊かな水辺や快適な生活空間の創造等まで期待されるようになった。そのため, 水の濁りや着色の程度を予測するために水中の有機物量を把握するだけでなく, 湖沼生態系の状態を示す新たな指標が期待されている。

我々のこれまでの研究では, 琵琶湖の COD の大部分が溶存有機物であり, COD の 6 割が難分解性有機物であること, 収支の推定から湖内由来の有機物が湖内全有機物の半分以上を占めることがわかってきた[1] [2]。これらの結果は, 湖水中の COD が流域からの直接の有機汚濁を反映しないだけでなく, 水中の酸素消費をしないものを測定していたことが明らかとなり, ますます COD の意味合いがあいまいとなっている。それゆえ, COD に代わる指標として,有機物の絶対量を把握できる全有機炭素（TOC）が提案されている[3]。

新たな有機物指標の是非を議論するには, COD のモニタリングデータの継続性も考慮して, COD と TOC の特性の違いを知っておく必要がある。COD_{Mn} は酸化力が不十分なことが知られているが, TOC に比較しての特性は定かとなっていない。そこで本研究では, COD が示す有機物の質を検討することを目的として, 琵琶湖およびその流域の河川や排水を用いて, COD と TOC との比較を行い, COD の検出特性を検討した。

2. 方法

滋賀県では, 1998 年から琵琶湖水質調査において, COD のモニタリングに加え, TOC の測定を開始した。加えて, 試水をガラス繊維ろ紙 Whatman GF/B（孔径 1 μm）でろ過して, 粒子態と溶存態に分画して P-COD, POC, D-COD, DOC 測定も開始している。そこで, 2014 年までの滋賀県が定点観測を行っている地点での湖水のモニタリングデータ[4]を用いて解析を行った。時系列解析は, 統計解析ソフト SPSS Trend によった。

TOC の測定は, ろ液を TOC 計にて, ろ紙で捕捉した POC を元素分析計により計測し, その和を TOC としている。COD, D-COD（ろ液の COD）は公定法にて測定し, P-COD は COD から D-COD を引いた差分である。

また，2007～2010 年度にかけて，琵琶湖，琵琶湖流域の主要な8つの河川水，流域内に存在する事業所排水，下水処理施設，水田および畑，宅地道路，山林他の面源等から各種排水より採取し，有機物の検討を行った[1] [2]。

3. 結果と考察

滋賀県定期調査での，湖水中の COD と TOC の時系列変動解析の結果を示した（図 1）。COD と TOC のトレ

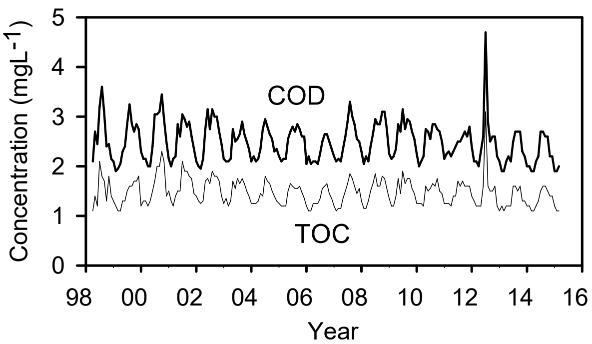

図 1 琵琶湖北湖17B点における COD, TOC の定期調査結果

ンドや季節変動は類似していた。しかし，湖水中の粒子態と溶存態別に TOC と COD の関係をみると，湖水の粒子態では TOC に対する COD の回帰直線の傾きが溶存態のそれより低かった（図 2）。粒子の酸化が不十分なため，P-COD の酸化率が低下することが考えられる。北湖，南湖の定点の平均値の COD/TOC は，湖水中の POC/TOC の比と相関があり，COD 値の分布には粒子態の有機物が影響している。

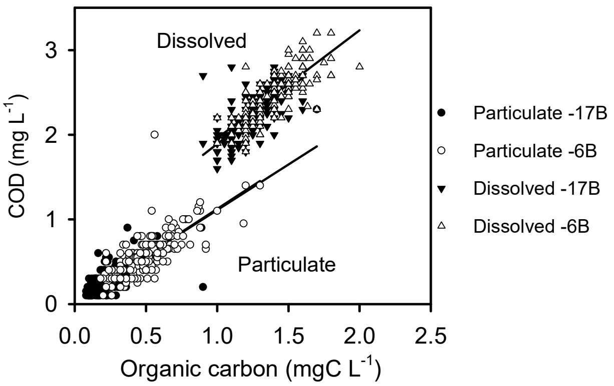

図 2 琵琶湖北湖(17B)，南湖(6B)における粒子態と溶存態別の TOC と COD の関係

また，河川や排水など様々な水環境調査の結果からも，COD/TOC の違いが確認された。河川水，特に渓流水で比が高くなる傾向がみられた。渓流水は比較的 COD で検出されやすい有機物を多く含むと考えられ，この特性は流域からの有機物負荷の算定に影響を及ぼす。滋賀県の第 5 期の湖沼計画での COD 負荷量推定から，本研究の COD/TOC を用いて TOC 負荷量を算出すると，流域からの有機物総負荷量に対する山林からの負荷量の割合が TOC で計算した場合に比べて 11%高い結果が得られた。したがって，COD で推定された有機物の負荷量計算は，実際の（TOC の）有機物負荷量より山林からの負荷を過大評価している。

COD/TOC が試料により違いが表れる原因には，粒子の他にも，有機物が低濃度での COD 測定時の過分解が考えられる。図2のグラフの DOC と D-COD の一次回帰式は D-COD = 1.36 DOC + 0.54 となり，X軸に切片がある。琵琶湖水中のアンモニア濃度は極めて低く，この切片を満たすほどの還元性物質は水中に存在しない。この切片は，低い有機物濃度では相対的に酸化剤の量比が増えてしまうため，過剰に酸分解が起こる COD 値の過大評価であると考えられた。市販の試薬（糖，アミノ酸）の COD 測定をすると，2 mgL-1 以下では，理論的な酸化率が上昇する傾向が見られる（図3）。

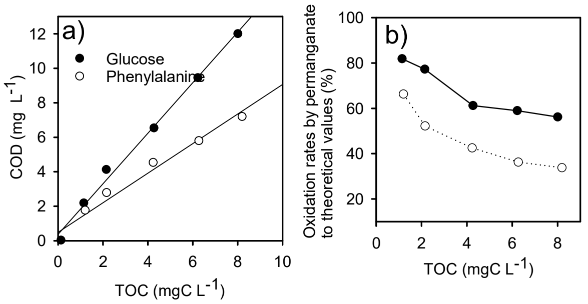

図 3 低濃度における COD の過剰酸化の影響

以上により，琵琶湖流域では，湖水や河川水の COD は有機物全体の半分程度を検出して，有機物濃度の分布を捉えているものの，1）有機物の性状や種類による検出感度の変化，2）低濃度での過大評価などの問題がある。それゆえ，琵琶湖水の COD 値は，有機物の汚濁レベルを見る上では差支えのない指標であるが，有機物の絶対量を推し量る指標としては問題がある。

4. 新たな有機物管理指標について

琵琶湖では，水質汚濁対策が進み，流域からの有機汚濁負荷は削減されてきた（図4）が，湖水中の有機物量には減少が見られない（図1）。湖内の内部生産によ

って有機物量が維持されているものと考えられる。

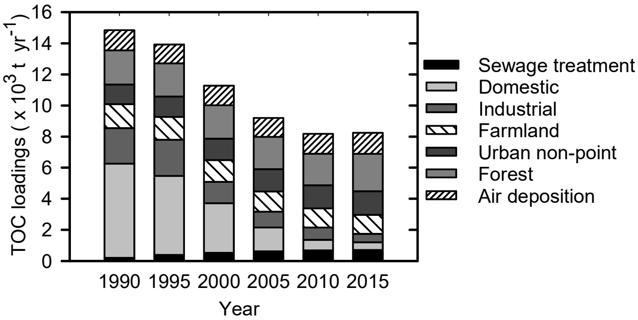

図 4 流域からの TOC 発生負荷量推定[5]

また，水質改善にもかかわらず，異臭味の発生による利水障害，外来魚の増加，水草の大量繁茂，在来魚類や二枚貝等の著しい減少等の問題が起きている。

このような生態系構造の不安定化に対して，生物の生息環境や産卵環境の保全はもとより，生態系を構成している生物群集相互やそれをとりまく環境との間での相互作用の円滑化が求められる。これらの相互作用を把握する鍵は生態系の物質収支の把握であり，生態系を構成している生物群集組成とその生産力の評価のための TOC 指標の導入による生態系の包括的な保全には欠かせない。

さらに水質監視では，河川は BOD，湖沼は COD と水域に応じた評価がなされているが，河川から湖沼までの生態系とそのつながりを含めた水環境を保全するためには，水域によらず統一的な指標で精度よく有機物動態を測る必要がある。それには TOC 指標による生産性の評価と合わせた有機炭素量のストックとフローの評価がふさわしいといえる。

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O3-25

伊豆沼沿岸部における底質有機物堆積機構の解析

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キーワード：底質有機汚濁，流速，連続観測，浅い湖沼

抄録

湖沼の水質は，河川や海域と比較して低い水準にある．この要因の一つには，底質に蓄積した有機物などの汚濁物質による内部負荷が挙げられる．そこで本研究では，湖沼における底質汚濁のメカニズムを踏まえた合理的な底質改善技術を提案するために，底質の有機汚濁化に着目し，底質有機物の堆積機構を明らかにすることを目的とした．加えて，流動が沈降や再懸濁と行った減少を通じて底質における有機物量を支配しているとの仮定のもとで，底質有機物量変動のシミュレーションモデルを構築し，流動制御が底質改善に寄与する可能性を検討した．その結果，底質有機物量は流動によって支配されており，底質からの再懸濁量が沈降量を上回ることで底質有機物量が減少することが示された．また，シミュレーションによって，実際の底質有機物量の変動を再現でき，底質有機汚濁が進んだ底質において流動を強化することによって，底質改善の効果が見込めることが示された．

1. はじめに

湖沼の水質改善を推進するためには，流域から河川を通じて流入してくる外部負荷対策と併せて，湖内での内部生産や底泥に蓄積した汚濁物質による内部負荷対策を行うことが重要である．一般的な内部負荷(底泥)対策としては，浚渫や覆砂が挙げられるが，こうした対策はその後の汚濁物質の再堆積・再溶出によって持続的な効果を得ることはできず， 底質環境に対する根本的な改善策にはならない．また，シードバンクの喪失等が懸念され，底生生態系への配慮に欠けることから，新たな底質改善技術が求められている．しかし，新たな底質改善技術の確立にあたっては，湖沼における底質汚濁のメカニズムを踏まえた合理的な方法を考案することが緊要である．そこで本研究では，内部負荷の大きな要因となっている底質の有機汚濁化に着目し，底質有機物の堆積機構を明らかにすることを目的とした．さらに，流動の強さと底質有機物量には負の相関があり，底質に蓄積している有機物は高等植物由来の難分解性有機物であるという既往の知見[1]から，流動が沈降・再懸濁といった現象を通じて底質における有機物量を支配しているという研究仮説のもと，流動制御が底質改善に寄与する可能性について検討した．

2. 研究手法

2.1 現地観測

流動と底質有機物に関するデータを現地観測により収集・解析することで，底質有機物堆積機構を明らかにすることを目指した．水環境の改善が進んでいない浅い湖沼として，宮城県北部に位置する伊豆沼を観測対象とした．伊豆沼は面積 3.69 km^2，最大水深 1.6 m，平均水深 0.76 m の浅い湖沼である．**図 1** に示す沿岸部の 4 地点で観測を行い，各地点で底質有機物量の指標として有機炭素(OC)含有率の測定，セディメントトラップによる沈降物質の捕集，流速や濁度等の連続観測(10 分毎に 1 秒間隔で 30 秒)を 2016 年 12 月から約 1 年間実施した．

2.2 底質有機物堆積機構の解析

下記の解析手法で流動が沈降・再懸濁を通じて底質における有機物量を支配するという研究仮説の検証を行った．

2.2.1 沈降・再懸濁と流動の関係

浅い湖沼においては，鉛直方向の SS 濃度は均一であると想定できるため，水中の SS 濃度の時間変化量は，式(1)で表せる[2]．

$$h\frac{dC}{dt} = E - S \tag{1}$$

ここに，h：水深(m)，C：SS 濃度(g/m^3)，t：時間(10 分間)，E：再懸濁フラックス(g/m^2/t)，S：沈降フラックス(g/m^2/t)である．SS 濃度に関しては，観測した濁度から検量線を作成して換算した．

式(1)において，左辺が正のときは再懸濁量が沈降量より大きいことを意味し，左辺が負のときは沈降量が再懸濁量より

図1 伊豆沼の全体図と観測地点

も大きいことを意味している．ここで，再懸濁量が沈降量を有意に上回る際の流速を明らかにするため，流速とSS濃度の相関が強かった期間に関して以下の解析を行った．

① 10 分毎の SS 濃度の平均値から，dC/dt を算出
② 10 分毎に 1 秒間隔で 30 秒間連続観測された流速データのうち最大のものを瞬間最大流速 u_{max} として抽出
③ 1 cm/s 毎に流速の閾値 u_c を設定
④ $u_{max} > u_c$ となる時の dC/dt について一群の t 検定
⑤ 95%信頼区間の下限値が正となる最小の u_c を算出

この時の u_c を，再懸濁量 ＞ 沈降量となる流速とした．

2.2.2 底質有機物量と流動の関係

底質有機物量と流動の関係を解析するため，本研究では既往の研究[1]と同様に，流動の指標として流速超過確率を用いた．

$$\mathrm{F}(V \geq V_c) = \frac{V_c\text{を超える流速の発生頻度}}{\text{流速データ数}} \tag{2}$$

ここに，F：流速超過確率，V_c：閾値とする任意の流速(cm/s)である．なお，流速データは流速超過確率を算出する期間中に，10 分間隔で 30 秒間連続観測した全てのデータ(1 個/s)を用いた．

2.3 底質有機物量変動のシミュレーション

底質有機物堆積機構のシミュレーションモデルを構築し，実際の湖沼における底質有機物含有率の変動を底質における物質収支で表現することを試みた．なお，本モデルは底質を有機物と無機物に分けて行い，簡易的に底質有機物含有率と底質 OC 含有率は等しいものとした．

2.3.1 底質有機物含有率

底質厚 0.1 m における有機物，無機物量の経時変化を求めるため，SS 濃度および流速の観測間隔である 10 分間隔で，以下の計算を行った．

$$Sed(O)_n = Sed(O)_{n-1} + S_O - \hat{E}_O \tag{3}$$

$$Sed(I)_n = Sed(I)_{n-1} + S_I - \hat{E}_I \tag{4}$$

ここに，$Sed(O)_0$：底質有機物量(g/m²)，$Sed(I)_0$：底質無機物量(g/m²)，$S_O(S_I)$：有機物(無機物)沈降フラックス(g/m²/t)，$\hat{E}_O$ ($\hat{E}_I$)：有機物(無機物)再懸濁フラックス(g/m²/t)である．なお，n：10分間隔の計算ステップ(n=1,2,3...)である．求めた底質有機物，無機物量から底質有機物含有率を算出した．

2.3.2 沈降フラックス

式(3)および式(4)における沈降フラックスの算出方法は以下のとおりである．

$$S_O = w_O(C_O - C_{O(min)}) \tag{5}$$

$$S_I = w_I(C_I - C_{I(min)}) \tag{6}$$

ここに，$w_O(w_I)$：有機物(無機物)沈降速度(m/s)，$C_O(C_I)$：有機(無機)懸濁物質量(g/m³)，$C_{O(min)}(C_{I(min)})$：計算期間中の有機(無機)懸濁物質量の最小値(g/m³)である．また，沈降速度の算出方法は以下のとおりである．

$$w_O = \frac{q_O}{C'_{O(ave)}} \tag{7}$$

$$w_I = \frac{q_I}{C'_{I(ave)}} \tag{8}$$

ここに，$q_O(q_I)$：セディメントトラップで捕集した有機物(無機物)沈降量の平均値(g/m²/t)，$C'_{O(ave)}(C'_{I(ave)})$：セディメントトラップ設置期間中の有機(無機)懸濁物質量の平均値(g/m³)である．なお，$C_O(C'_O)$は SS 濃度に懸濁物の OC 含有率を乗じて求めた．$C_I(C'_I)$については，SS 濃度と $C_O(C'_O)$との差から求めた．

2.3.3 再懸濁フラックス

式(3)および式(4)における再懸濁フラックスの算出方法については，以下の式を用いて，水中懸濁物質の物質収支から再懸濁フラックスを算出した．

$$E_O = S_O + h\frac{dC_O}{dt} \tag{9}$$

$$E_I = S_I + h\frac{dC_I}{dt} \tag{10}$$

次に，再懸濁フラックスを流速の関数として表現するため，$E_O(E_I)$と流速について回帰分析を行った．

$$\hat{E}_O = \alpha_O v \tag{11}$$

$$\hat{E}_I = \alpha_I v \tag{12}$$

ここに，$\alpha_O(\alpha_I)$：各地点における再懸濁係数，v：瞬間最大流速(cm/s)である．

3. 結果

3.1 現地観測

平均底質 OC 含有率は，Stn.A：7.0 %，Stn.B： 2.5 %，Stn.C：0.5 %，Stn.D：3.3 %であった．また，年間の推移については観測期間全体を通じて目立った季節的な傾向は見られなかった．**図 2** に代表的な地点における底質，沈降物質，水中懸濁物質の OC 含有率の時系列データを示す．各地点ともに水中懸濁物質 ＞ 沈降物質 ＞ 底質の順に有機物量が多かった．

3.2 底質有機物と流動の関係解析

図 3 に各地点の流速超過確率と底質 OC 含有率の関係を示す．任意の閾値を 1 cm/s 刻みで 1～10 cm/s まで変化

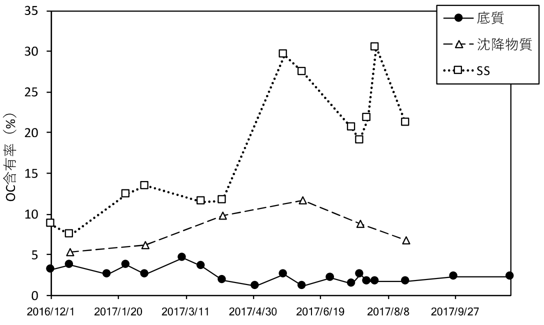

図2 Stn.Bにおける底質，沈降物質，SSのOC含有率

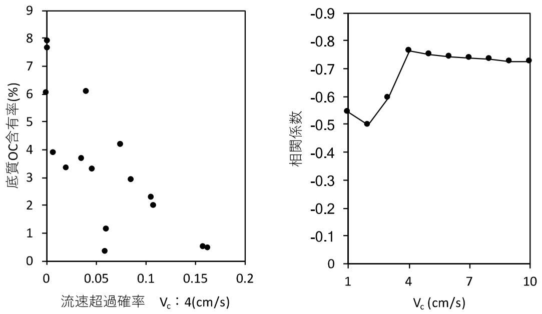

図3　底質OC含有率と流速超過確率の関係

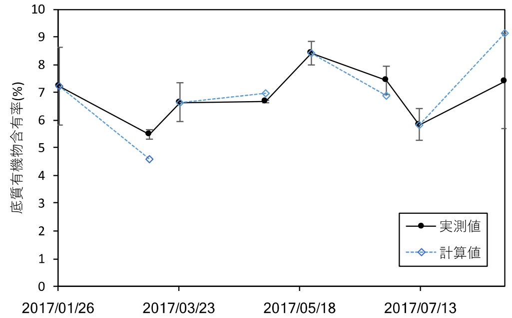

図4　Stn.Aにおける実測値と計算値の比較

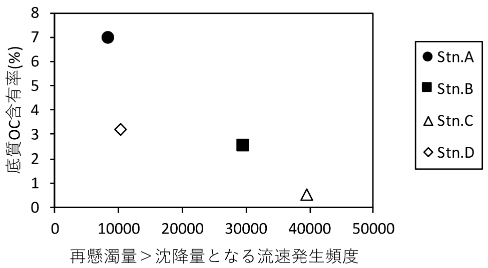

図5　再懸濁量 > 沈降量となる頻度と底質OC含有率の関係

させたところ，4 cm/s 以上の流速発生頻度と底質 OC 含有率の間に最も強い負の相関を得た(r＝- 0.76, $p<0.01$)．また，各地点で底質の再懸濁量が底質への懸濁物質の沈降量を上回る流速の条件を解析した結果，4～6 cm/s を超える流速が観測された時，有意に再懸濁量が沈降量を上回っていることがわかった($p<0.05$)．

3.3 底質有機物量のシミュレーション

図 4 に Stn.A における底質有機物含有率の計算結果と実測値の比較を示す．再懸濁量を流動に比例する関数として与えることで，底質有機物含有率の増減を再現することができた．

4. 考察

沈降・再懸濁と流動の関係を解析した結果，各地点で，4～6 cm/s 以上の流速が発生した際に再懸濁量が沈降量を上回っており，4 cm/s を超える流速発生頻度が多くなれば底質有機物量が減少することが分かった．さらに，底質 OC 含有率と流速超過確率の間で，負の相関のピークが現れた閾値が 4 cm/s であり，それ以上の閾値においても同程度に強い負の相関を得たことから，再懸濁量が沈降量を上回るとき，底質の有機物量が減少すると考えられる．したがって，研究仮説のように流動が沈降・再懸濁を通じて底質における有機物量を支配していることが示唆された．

再懸濁が卓越するときに底質有機物量が減少する理由は，SS，沈降物質，底質の OC 含有率の大小関係(**図 2**)から説明できる．セディメントトラップで捕集した沈降物質は，主に底質が巻き上がり，再沈降したものであると考えられる．その理由として，セディメントトラップの開口部は 20 mm であり，それより大きな物体は捕集されないこと，伊豆沼の水深は浅いため，沈降の過程で植物の枯死体等が細かくなる可能性は低いこと等が挙げられる．これを踏まえた上で SS，沈降物質，底質の OC 含有率の大小関係を比較すると，概ね SS＞ 沈降物質 ＞ 底質という関係にあったことより，底質の再懸濁時には有機物が選択的に再懸濁していると考えられ，その一部は再懸濁したまま水中に残存すると予想される．したがって，再懸濁量が沈降量を上回る頻度が低い地点(Stn.A)では，底質有機物含有率が，湖内で生産された高等植物の枯死体等の有機物含有率に近づき，再懸濁量が沈降量を上回る頻度が高い地点(Stn.C)では，底質からの有機物の選択的な離脱が起こるために，有機物含有率が低くなるものと考えられる．**図 5** に，各地点における再懸濁量＞沈降量となる流速の発生頻度と平均底質 OC 含有率を示す．

底質有機物量のシミュレーションでは，Stn.A で底質における有機物・無機物の収支を計算することで有機物含有率の変動を再現することができた．また，再懸濁フラックスは流動に比例する関数として与えることで良好な再現性を得ており，再懸濁の卓越が底質有機物量を低下させることから，Stn.A のような底質有機物含有率の高い場では，流動の強化によって底質改善の効果が見込めることが示された．

5. まとめ

浅い湖沼においては，流動が沈降，再懸濁を通じて底質における有機物量を支配していることが示唆された．底質の再懸濁時に，有機物は底質から選択的に離脱するため，再懸濁量が沈降量を上回る頻度が高まるほど底質有機物量は少なくなる．また，底質有機物量の変動は底質における有機物，無機物の物質収支を計算することで再現できた．再懸濁量を流速に比例する関数として与え，良好な再現性を得たことから，流動の強化が底質有機汚濁の改善に有効であることが示された．

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O3-26

Nutrients and molar C:N:P ratios in surface sediments of the Songkhla Lagoon system in southern Thailand

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Key words: sediment nutrients, coastal lagoon, Songkhla Lagoon system, Thale Sap, Songkhla Lake

ABSTRACT

Information pertaining to temporal and spatial variations in nutrients concentrations in the surface sediments of the Songkhla Lagoon system is limited. Therefore this study aimed to examine the proportion of silt and clay and total carbon, nitrogen and phosphorous concentrations in the surface sediments from the windward and leeward sites of Thale Sap and Thale Sap Songkhla in the Songkhla Lagoon system between August 2017 and March 2018. Samples taken from leeward shores exhibited significantly higher proportion of silt and clay. The sediments of Thale Sap were characterized by higher concentrations of carbon and nitrogen, owing to the colonization of aquatic macrophytes within this lake. The phosphorous concentrations were relatively high in the sediments of Thale Sap Songkhla, owing to the proximity of this lake to discharge sources such as urbanization, industries, and human settlements. The sediments of Thale Sap had a relatively higher molar C:N:P ratios (437:43:1) than that of the sediments of Thale Sap Songkhla (233:23:1).

1. INTRODUCTION

Coastal Lagoons are transitional zones located between fresh- and salt-water nvironments and, have high ecological and socio-economic values. The Songkhla Lagoon system in southern Thailand is a shallow and well-oxidized tropical coastal lagoon that supports fisheries for shrimp, crab, and various fish species, including intensive aquaculture of barramundi (*Lates calcarifer*)(1). As other coastal lagoons, the Songkhla Lagoon system is also subject to strong anthropogenic pressure associated with urbanization, industrialization, artisanal fishing, and recreation(2). In 2005, a study reported evidence of eutrophication in the Thale Luang and Thale Sap lakes of the lagoon system(3); however, this study was limited to estimating chlorophyll *a* concentrations, with no information regarding excess nutrient concentrations.

Due to the shallowness of the Songkhla Lagoon, wind direction and velocity can force the sediments to be resuspended, redistributed, and redeposited within the system. During these processes, the coupling nutrients in the overlying waters along with microbial-mediated nutrients, the associated pore water, organic detritus, and inorganic particles associated with geochemical processes are also released within the system(2). Studies investigating nutrient concentrations within the lagoon system are critical to discerning changes in the biological behavior of the lagoon; however, such information concerning the distribution and retention of sediment nutrients in the Songkhla Lagoon is currently lacking.

The objectives of this study are to 1) characterize the temporal and spatial patterns in the nutrient (C, N, and P) concentration and 2) analyze the relationships among C, N, and P concentrations in the surface sediments of the Songkhla Lagoon system. To my knowledge, this study is the first to report the temporal and spatial patterns in the nutrient concentration in the sediments of the Songkhla Lagoon system. The results of this study will aid in the broader understanding of the fate of nutrients within this lagoon system.

2. MATERIALS AND METHODS

Study sites

The Songkhla Lagoon system, also known as Lake Songkhla (ASI-02)(1), is a large shallow water body located on the east coast of Thai's Peninsula in southern Thailand situated between 7° 08′N and 7° 48′N and between 100° 07′E and 100° 35′E. The lagoon system covers approximately 1,082 km^2, comprises four distinct parts: Thale Noi, Thale Luang, Thale Sap, and Thale Sap Songkhla, and exhibits three water regimes: fresh, brackish, and salt water arranged from north to south, respectively, with the southern end connected to the Gulf of Thailand. Within the lagoon system, salinity ranges from 0 to 34 psu in Thale Sap Songkhla to almost zero in Thale Noi. The shores of the Songkhla Lagoon system comprise a mixture of urban areas, fishing villages, shrimp ponds, seasonally flooded forests, paddy fields, and mangrove forests. The submerged vegetation in Thale Sap is dominated by *Najas marina*, *Najas minor*, *Hydrilla verticellata*, and *Potamogeton malayanus*. Conversely, the growth of submerged macrophytes is limited. In both Thale Sap and Thale Sap Songkhla, the substratum is primarily mud.

Sampling methods

Bed sediments were collected at monthly intervals between August 2017 and March 2018 from eight stations located along the shores of the lakes Thale Sap and Thale Sap Songkhla. In each part of the lake, there were two stations classified as windward and the others were leeward with two replicates at each site. Bed sediments representing recent deposits were collected using a plastic spade by scooping from the upper 5 ± 5 cm of the surface sediments at a water depth of approximately 50–150 cm. The sediments were then packed and sealed in plastic containers and transferred to the laboratory of Faculty of Science, Prince of Songkla University, where they were air-dried in an oven at 60°C.

Data collection

Sediment samples were passed through a 0.0625-mm standard mesh sieve to separate the silt and clay fractions from the sand fractions. The total carbon (Tot-C) and total nitrogen (Tot-N) concentrations in the sediment samples from each replication were determined by combustion of the dried and processed surface sediments in a CHN analyzer. The total phosphorus (Tot-P) concentration was determined using ICP-AES.

Data analysis

Nutrient concentrations in the sediment samples were compared using a three-way analysis of variance. The sampling month, lake, and shore were considered as independent variables and the proportion of silt and clay and the concentrations of C, N, and P as dependent variables.

3. RESULTS

The sediments from both Thale Sap and Thale Sap Songkhla are dominated by silt and clay. The sediment samples from both lakes predominantly comprised grains <63 μ (51.77 %–91.87% by weight). There was no significant difference in the proportion of silt and clay within the sediments in both lakes. At closer distribution patterns, there was a significant difference in the proportion of silt and clay among sampling months and between both shores (Table 1). The proportion of silt and clay was higher in sediment samples from leeward shores than in those from windward shores (Fig. 1A). The sediment samples from Thale Sap and Thale Sap Songkhla had significantly different carbon (Tot-C) and nitrogen (Tot-N) concentrations (Table 1). The concentrations of C and N in the sediments of Thale Sap were significantly higher than that in the sediments of Thale Sap Songkhla (Figs. 1B and 1C). Similar patterns were noted across sampling months, where Tot-N concentrations were consistently higher in the sediments of Thale Sap than in the sediments of Thale Sap Songkhla (Fig. 1D). During the rainy season (September–January), the sediments of Thale Sap Songkhla had significantly higher phosphorous concentrations than that in the sediments of Thale Sap, while no different for the rest (Table 1 and Fig. 1E). The highest molar C:N:P, C:P, and N:P ratios were found in the sediments of Thale Sap (Table 1). However, there were no significant differences in the C:N:P and C:N ratios between the two lakes.

4. DISCUSSION

A high proportion of silt and clay measured in sediments from the leeward shores of both Thale Sap and Thale Sap Songkhla suggests that the wind-driven waves that dominate the windward shore keep the silt and clay resuspended, rendering the water turbid during periods of high wind [2,4]. These alterations in the present study were governed by the seasonal northeast monsoon wind, a primary force that influences several coastal lagoons. Particle size of the sediments may therefore particularly small on the leeward and increase toward the windward shore.

The relatively high nutrient concentrations, particularly those of carbon and nitrogen, measured in the sediments of Thale Sap suggest that the aquatic macrophytes that inhabit Thale Sap strongly contribute to the nutrient enrichment of the lake sediment. In general, macrophyte detritus alters sediment stoichiometry by accumulating more C relative to N and P, making the sediments of Thale Sap more suitable for decomposers when compared with the sediments of Thale Sap Songkhla. The importance of macrophytes is not restricted to the sediment. They also influence the composition of suspended POM and thus, represent an important coupling between benthic and pelagic habitats within this lagoon. High C:P ratios measured in the sediments of Thale Sap and some stations within Thale Sap Songkhla may also reflect the influence of the macrophytes that are abundantly present in this region. It is interesting to note that there were relatively low nutrient concentrations measured in the sediments of Thale Sap Songkhla, however, the annual catches for penaeid prawns were obviously high suggest that nutrients that originate from autochthonous in Thale Sap may then be transferred to Thale Sap Songkhla. The relatively high phosphorus concentration in the sediments of Thale Sap Songkhla may be associated with urbanization, industrial activities, and the high population densities of the Songkhla and Hat Yai cities.

Table 1 Percentage of Silt & Clay, Nutrient concentrations (Mean ± SE) Total Carbon, Total Nitrogen and Total Phosphorus (μmol g^{-1}), molar C:N:P, C:N, C:P and N:P ratios for sediment nutrients in Thale Sap(TS) and Thale Sap Songkhla (TSS) of the Songkhla Lagoon system

Locality	Silt&Clay Fraction	Total Carbon (μmol g^{-1})	Total Nitrogen (μmol g^{-1})	TotalPhosphorus (μmol g^{-1})	C:N:P	C:N	C:P	N:P
TS	68.12±1.16	1599.1±100.31	157.12±17.48	3.66±0.56	437:43: 1	10	437	42.9
st 1	67.70±2.61	1191.4±51.82	131.73±6.23	1.23±0.75	967:107:1	9	967	107.1
st 2	68.89±2.58	1369.6±114.67	141.52±8.55	2.99±0.82	458:47:1	10	458	47.3
st 3	64.12±1.56	1795.4±295.35	136.46±10.22	4.08±1.05	440:33:1	13	440	33.4
st 4	72.32±1.88	2039.9±180.18	148.87±10.65	6.18±1.36	330:24:1	14	330	24
TSS	70.11±1.14	1112.1±58.02	105.46±4.54	4.77±0.76	233:22:1	11	233	22.1
st 1	76.50±1.94	1449.0±122.69	104.64±4.35	2.50±0.70	580:42:1	14	580	41.9
st 2	69.43±1.23	797.0±61.68	93.77±7.08	6.30±1.56	126:15:1	8	126	14.9
st 3	61.32±1.13	1403.6±69.70	138.76±9.52	7.01±2.07	200:20:1	10	200	19.8
st 4	73.14±1.39	776.4±27.34	83.83±7.29	3.39±1.20	229:25:1	9	229	24.7

5. CONCLUSION

In this study, sediment nutrient concentrations (Tot-C and Tot-N) were higher in Thale Sap than in Thale Sap Songkhla. Thus, within the Songkhla Lagoon system, Thale Sap is an important organic source and sink, contributing to the nutrient concentration of Thale Sap Songkhla. It is interesting to note that the C and N concentrations measured in the present study in surface sediments of both Thale Sap and Thale Sap Songkhla are higher than the concentrations reported from the Songkhla Lagoon system's terrestrial catchments[(5)]. This suggests that the excess nutrients driving eutrophication in the Songkhla Lagoon system are generated by sediment resuspension processes coupled with the biological activities of microorganisms and hydro-biogeochemical features within the system.

Further studies

1) Investigation of the role of dominant aquatic macrophytes as a source of organic matter to nutrient cycles in the Songkhla Lagoon system.
2) Examination of bacterial metabolism and growth efficiency associated with nutrient availability in the lagoon system.
3) Assessment of nutrient concentrations, biomass, and detritus from aquatic macrophytes within the lagoon system in relation to salinity and rainfall.

Acknowledgment

The present study is a part of a research on Distribution patterns and variability in abundance of postlarvae and juveniles of *Metapenaeus* spp. for fishery status and management guidelines in Thale Sap Songkhla, southern Thailand, supported by a grant SCI600364S from Prince of Songkla University.

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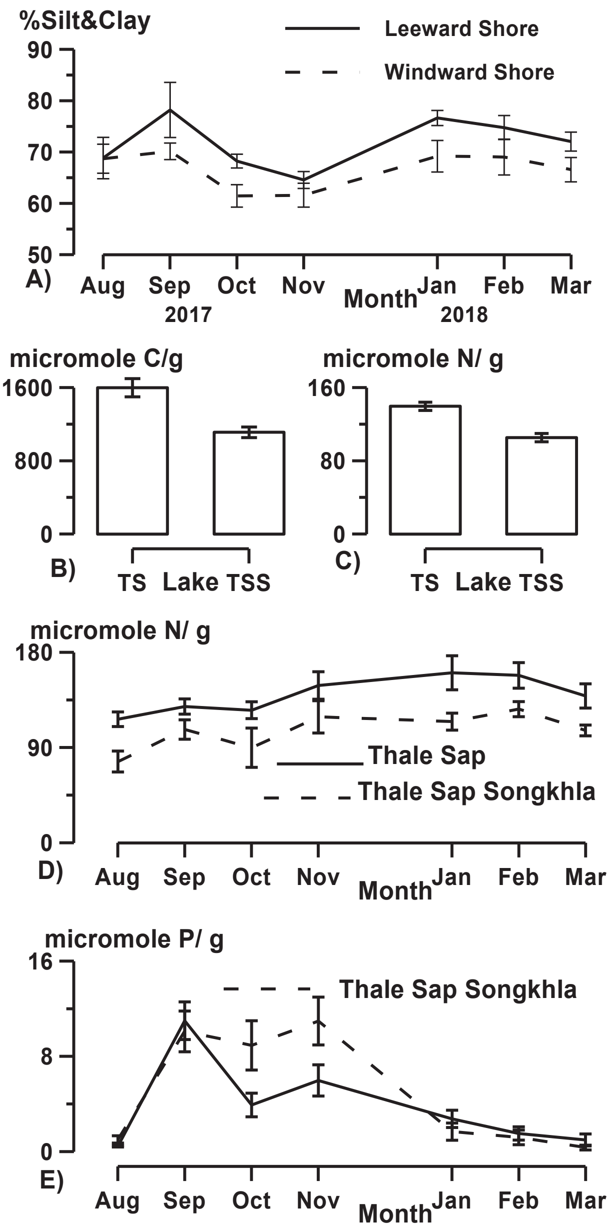

Figure 1. Temporal and spatial variations in nutrient sediments of Songkhla Lagoon system, southern Thailand.

A) **Proportion of silt and clay between windward and leeward shores, between Aug 2017 and Mar 2018**
B) **Tot-C Concentration in nutrient sediments between Thale Sap(TS) and Thale Sap Songkhla(TSS)**
C) **Tot-N Concentration in nutrient sediments between Thale Sap(TS) and Thale Sap Songkhla(TSS)**
D) **Tot-N Concentration in nutrient sediments from Thale Sap(TS) and Thale Sap Songkhla(TSS) between Aug 2017 and Mar 2018**
E) **Tot-P Concentration in nutrient sediments from Thale Sap(TS) and Thale Sap Songkhla(TSS) between Aug 2017 and Mar 2018**

O3-27

Phytoplankton Communities and Water Quality Characteristics in Lake Paldang, South Korea

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Keywords: Lake Paldang, phytoplankton, environmental index, trophic state, principal component analysis

ABSTRACT

The present study carried out the temporal characteristics and variation of phytoplankton diversity, composition and their abundance with water quality using statistical techniques in Lake Paldang, South Korea. A total of 91 species of phytoplankton were identified from the Lake Paldang in 2016, belonging to 6 functional groups i.e., Chlorophyta(35 species), Bacillariophyta(34), Cyanophyta(11), Ochrophyta(5), Cryptophyta(4), and Miozoa(2). Throughout the study period, the occurrence of most dominant species was observed from Bacillariophyta(*Stephanodiscus hantzschii* and *Cyclotella atomus*). The performed statistical analysis on phytoplankton species, the evenness and Shannon-wiener diversity index were found to be higher in winter than those of spring. PCA applied to compare the compositional patterns among the analyzed water samples, identified and four factors accounting for almost 84.4% of the total variation representing water compound concentration are mainly related to point and non-point source as well as natural process. We therefore suggest wise management of anthropogenic activities in the catchment of Lake Paldang and their tributaries.

1. INTRODUCTION

The lakes and rivers represent the major source of water used for human consumption culture irrigation, industrial purposes and drinking water. Water resources require information about water quality and its variability. Phytoplankton is the primary source of a food chain, which contributes to the major fishery resource around the world. They are responsible for the formulation of a biological community and regulate the food web[1,2].

Phytoplankton community structure, composition and species diversity in aquatic ecosystem are determined by several physic-chemical parameters[3]. Temporal variations in phytoplankton distribution are widely affected by the hydrochemical and physical factors such as temperature, pH, TSS and nutrients. Phytoplankton reacts rapidly to environmental changes and are regarded proper indicators of water quality and trophic conditions owing to their short generation time and fast population renewal[4]. Freshwater communities are very sensitive to environmental variables. Local processes interact to produce phytoplankton patterns of species abundance and diversity in a freshwater ecosystem[5].

Recently, the multivariate statistical approach is popular form a better understanding of water quality and aquatic status, in view of their ability to treat large volume of spatial and temporal data from a variety of monitoring sites. In the scientific published papers, principal component analysis(PCA) was used for this sort of studies in that they are capable to assess temporal variations in water quality and to identify potential sources of water pollution[6,7].

The present study aims to show characteristics and variation of phytoplankton diversity, composition and their abundance in Lake Paldang, South Korea. Temporal variations in different physic-chemical factors and environmental indices affect the phytoplankton growth have been discussed.

2. METHODS & MATERIALS

Study location

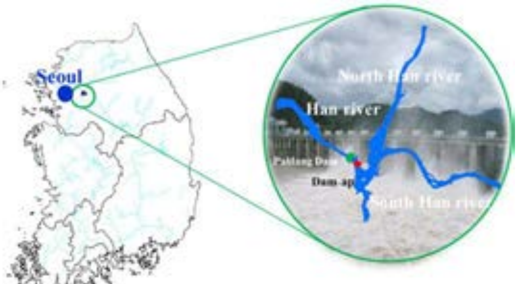

Fig. 1. Location of Lake Paldang with sampling site

Lake Paldang is constructed in 1973 for the purpose of hydroelectric power generation and creating a drinking water source an artificial dam reservoir (Fig.1).

The location is in the central part of the Korean Peninsula(37°29´ N, 127°26´ E), serving as a major source of drinking water for more than 25 million people in the Seoul, South Korea. The catchment area of the lake is as much as 23,800 km^2, which corresponds to a quarter of the total area of South Korea(100,032 km^2).

Sampling and water quality analysis

For qualitative and quantitative studies of phytoplankton, 2 liters of water sample was taken from near surface and fixed with few drops of acid Lugol's iodine solution(1 ~ 2%). After the settling and siphoning procedure, 1 ml of aliquot of the sample was taken in a Sedgewick-Rafter counting cell in duplicate under the inverted microscope(Eclipse Ni-E, Tokyo, Japan) for identifying and counting phytoplankton cells.

Water temperature, dissolved oxygen(DO), pH, conductivity(EC), were measured in situ with a portable multi-prove(YSI EXO, Yellow Springs, OH, USA). 2 L water samples were also collected from mixture of subsurface(0.5 m) and middle(10 m) and bottom(17 m) level for analysis at site, including the following parameters; total phosphorus(TP), total nitrogen(TN), nitrate nitrogen(NO_3-N), ammonia nitrogen(NH_3-N), five-day biochemical oxygen demand(BOD_5), chemical oxygen demand(COD_{MN}), chlorophyll-*a*(Chl-*a*), total suspended solid(TSS). All samples were analyzed according to Korean Standard Method for Examination of Water Quality[8].

Environmental index and statistical analysis

Diversity index(H\`), evenness(J\`) and richness index(S) were used to calculate community metrics of phytoplankton[9]. The trophic state index(TSI) is obtained by incorporating data for these three parameters into the corresponding Carlson(1977) equation[10]. In order to facilitate consistent evaluation of the dataset of these multiple variables monitored during various periods, multivariate analyses of the water quality dataset were performed through principal component analysis(PCA). Statistical computations in this study were made using IBM SPSS20.

3. RESULTS & DISCUSSION

Species composition and diversity of phytoplankton

A total of 91 phytoplankton species belonging to 6 phyla were recorded in 2016, including Bacillariphyta, Cyanophyta, Chlorophyta, Cryptophyta, Ochrophyta and Miozoa. In terms of species number, 35 species was group of Chlorophyta, followed by Bacillariphyta with 34 species, Cyanophyta with 11 species and other algal groups were total 11 species. Among them, 57 species in 6 phyla were collected in summer while 28 species from 5 phyla were observed in winter. Although the highest number of species was Chlorophyta the Bacillariphyta was the most dominant group with the high abundance(141 × 10^3 cells L^{-1}) in all seasons, except of August(Cyanophyta) and December(Cryptophyta). The variation of cell number and relative abundance of each phytoplankton group were illustrated in Fig. 2.

The species richness(S), and Shannon-Wiener diversity(H\`) were generally low in the Lake Paldang, which ranged from the highest 57 species in summer to the lowest 28 species in winter, and diversity varied between 0.40 and 2.70. Seasonally, species diversity and evenness were much higher in winter(average H = 2.08; J\` = 0.79) than those of spring(average H = 1.73; J\` = 0.58). The richness(S) were much higher in spring(average S = 2.13) than that of autumn(average S = 1.54). The low evenness in spring indicated that the phytoplankton community was dominated by a few species, coinciding with the observation of the blooms of *Stephanodiscus hantzschii* and *Cyclotella atomus* in the study area during spring sampling.

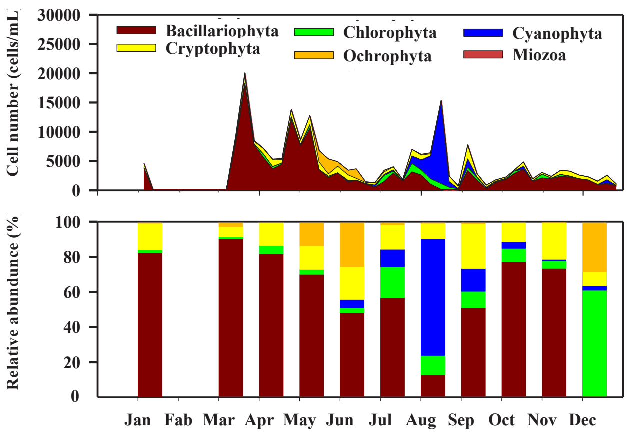

Fig. 2. The variation of cell number and relative abundance of each phytoplankton group

Source identification of monitored variables and TSI

The PCA based on the correlation matrix was performed to understand the underlying relationships between the water quality variables of Lake Paldang and to identify their characteristics. The Kaiser's criterion was used to determine the number of PCs to retain[11]. The scree plot(Fig. 3) showed the sorted eigenvalues from large to small as a function of the PC number. Loadings of four retained PCs are expressed in Table 1.

The first factor(PC1), according 40.82% of the total variance, showed high positive loadings of NO_3-N, DO, T and NH_3-N, and high negative loading of TEMP. The second factor(PC2) explained 19.31% of the total variance. It had strong positive loading on COD, pH, BOD and Chl-*a*. The third factor(PC3), explained 15.30% of the total variance, contains a large positive loading on TP and SS. The fourth factor(PC4) accounting for the lowest total variance(9.01%), has a strong positive loading on EC. This contamination is due to a mixed source including natural processes and anthropogenic activities. The average values of TSI(SD), TSI(TP) and TSI(Chl-*a*) are 51.59, 48.66 and 54.10 respectively, showing in the category of mesotrophic/eutrophic.

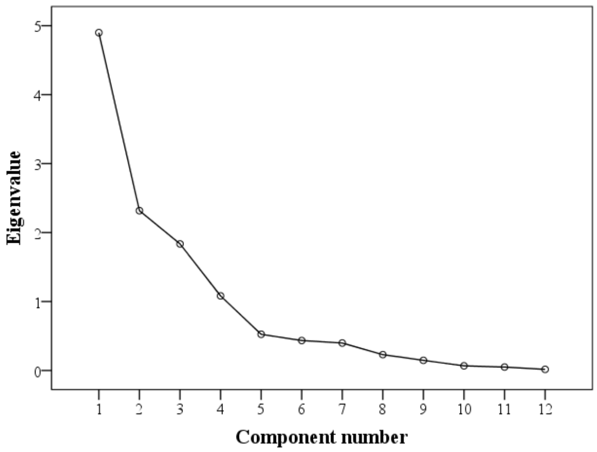

Fig. 3. Scree plot of the eigenvalues.

Table 1. Loading of experimental variables on principle components for the whole datasets

	PC1	PC2	PC3	PC4
Temp	**-0.933**	0.113	0.038	0.108
NO_3-N	**0.824**	0.313	0.267	0.187
DO	**0.821**	0.402	-0.223	-0.078
TN	**0.801**	0.320	0.323	0.300
NH_3-N	**0.607**	-0.289	0.143	0.521
COD	-0.164	**0.820**	0.347	0.127
pH	0.152	**0.789**	-0.335	0.283
BOD	0.384	**0.756**	0.222	-0.025
Chl-*a*	0.294	**0.557**	0.450	-0.141
TP	-0.092	0.039	**0.951**	-0.099
SS	0.243	0.178	**0.917**	0.017
EC	0.091	0.234	0.007	**0.893**
Eigenvalues	4.899	2.317	1.836	1.082
% of variance	40.822	19.312	15.297	9.014

4. CONCLUSIONS

This paper summarized the weekly variations of physic-chemical parameters and their influences on phytoplankton community of Lake Paldang with environmental indices and statistical data output in 2016. The phytoplankton community was found to belong to 6 groups, with Bacillariophyta dominating except of August and December. The indices of phytoplankton species diversity(H`) and evenness(J`) in winter(av. H = 2.08; J` = 0.79) were higher than spring(av. H = 1.73; J` = 0.58). Several trophic state indices and water quality parameters have been applied an overall analysis pointed out mesotrophic/eutrophic characteristics. As a result of PCA, water pollutants are mainly in associated with both natural processes and anthropogenic activities.

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O3-29

ヨシ根圏におけるビスフェノール類の分解に関する検討

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キーワード:ビスフェノール類, ヨシ, 根圏微生物, 分解

抄録

ビスフェノール A (BPA) やビスフェノール F (BPF) 等のビスフェノール類 (BPs) は工業的に重要な化学物質であるが, 内分泌攪乱性を有しており, 水域への排出に伴う生態系への悪影響が懸念されている。他方, 世界各地の湖沼などの水域や湿原に生息するヨシは, 水質浄化機能や生物多様性の保全機能等を有することが知られている。そこで本研究では, ヨシと根圏微生物の存在下における BPA 及び BPF の挙動について検討した。その結果, 特に BPA においては, ヨシが存在しない場合と比べて, ヨシと根圏微生物の存在下で分解が促進されることが確認された。また, 微生物群集解析の結果, 分解の進行とともに BPs 分解微生物と考えられる特定の微生物の集積が観察され, さらに, ヨシ植栽により微生物群集の多様性が高まることも確認された。これらの結果から, ヨシの植生は水域における BPs の分解, 及び微生物生態系の多様性維持にとって重要な意義を有するものと示唆された。

1. はじめに

ビスフェノール A (BPA, 図 1(a)) はポリカーボネート樹脂やエポキシ樹脂などの原料として工業的に幅広く使用されている重要な化学物質であるが, 内分泌攪乱化学物質であることが知られている。近年では, 様々な水環境中において検出されており, 水域生態系への悪影響が懸念されている。そこで, BPA の代替物質としてビスフェノール F (BPF, 図 1(b)) 等のビスフェノール類 (BPs) の使用が進められているが, それらの中にも内分泌攪乱性を有するものが存在しており, BPA と同様に水環境汚染に伴う生態系への悪影響が懸念されている[1]。このため, 水環境中における BPs の消長について詳細に理解することが必要である。他方, ヨシ (*Phragmites australis*) は世界各地の水域や湿原に生息しており, その群落には水質浄化機能や生物多様性の保全機能等が期待されている。既往研究では, ヨシの根圏に BPs を含む芳香族化合物の分解微生物が選択的に集積することも明らかにされている[2,3]。そこで本研究では, ヨシと根圏微生物の存在が BPs の挙動に及ぼす影響について検討した。

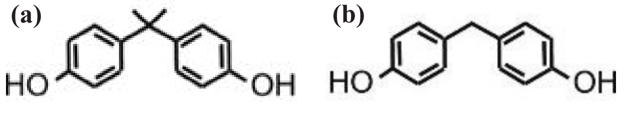

図 1　BPA (a) と BPF (b) の構造式

2. 方法

2.1 実験に使用したヨシ及び微生物群集

ヨシは, 種子をエタノール, 次亜塩素酸ナトリウム, 滅菌脱イオン水で洗浄した後, 滅菌改変 Arnon & Hoagland (A&H 培地) で 8～12 週間栽培したものを使用した。また, 大阪大学内にある犬飼池より採取した池水を孔径 10 μm のフィルターでろ過して夾雑物質を除去した後, 遠心分離 (10,000×*g*, 4℃, 10 分) によって水中の微生物群を回収し, A&H 培地で 2 回洗浄し, A&H 培地に再懸濁させたものを微生物懸濁液として実験に使用した。

2.2 BPs 除去実験

BPA または BPF を 20 mg/L となるように添加した A&H 培地 100 mL を植物培養瓶に分注して実験系を作成し, 人工気象器内 (28℃, 16 h-light/8 h-dark) に静置して実験を行った。実験系は, ヨシと微生物懸濁液の両者とも植栽／植種しない系 (A), 微生物懸濁液のみを植種した系 (B) , ヨシを 2 株植栽した系 (C) , (B) にヨシを 2 株植栽した系 (D) の 4 種類を作成した (各々1 連)。

2.3 分析方法

実験期間中は, 適宜, 各実験系から 1 mL の試料を採取し, 2N HCl を 10 μL 滴下して生物反応を停止させた後, 孔径 0.45 μm のフィルターでろ過し, HPLC を使用して BPs 濃度を測定した。ただし, 実験期間中の水の蒸発による実験結果への影響を避けるため, 試料採取時には事前に滅菌超純水を用いて蒸発した水量を補完した。また, 実験系 B および D については, 実験開始時, 実

験中，終了時の試料から微生物 DNA を抽出し，16S rRNA 遺伝子を標的とした Terminal Restriction Fragment Length Polymorphism (T-RFLP) 法を用いて微生物群集構造を解析した。

3.　実験結果および考察方法

3.1　BPsの分解挙動

各実験系における BPA，BPF の濃度変化を図 2 に示す。BPA は，ヨシと微生物群集の両方を含む実験系 D，及びヨシのみを含む実験系 C において減少し，分解の進行が確認された (図 2(a))。実験系 B，C，D における結果から，BPA は環境中の微生物のみでは分解されにくく，ヨシと微生物の共生により分解が促進されることが示唆された。また，実験系 D で明確な分解が確認されたことから，BPA を添加した微生物懸濁液で 2 週間栽培したヨシを植え継ぎ，さらに 2 週間栽培したヨシを 2 株植栽した系を実験系 E として作成し，同様の実験を行った。その結果，実験系 E では実験系 D よりも BPA の減少速度が向上したことから (図 3)，実験系 E では事前の培養によってヨシ根圏に BPA 分解菌が集積されたことにより，分解が促進されたものと考えられた。また，BPA の分解に伴う代謝物の生成についても検討を行ったところ，Spivack ら[4]が BPA の生分解代謝物として報告している 4-ヒドロキシ安息香酸 (HBA)と 4-ヒドロキシベンズアルデヒド(HBAL) または 4-ヒドロキシアセトフェノン (HAP) の生成が確認され，本研究の実験系においても，BPA が既知の微生物分解経路によって分解されたものと示唆された。

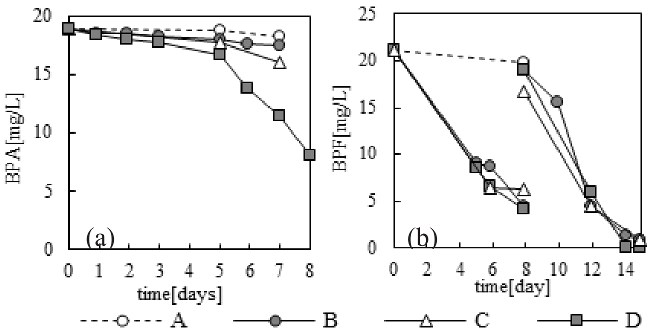

図 2　各実験系における BPA (a)，BPF (b) の濃度変化

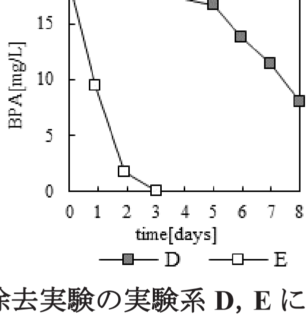

図 3　BPA 除去実験の実験系 D，E における濃度

他方，BPF は，微生物群のみを含む実験系 B，ヨシのみを含む実験系 C，ヨシと微生物群を含む実験系 D において類似の減少傾向を示した (図 1(b))。しかし，実験系ごとに生成する中間代謝物は異なった。

3.2　微生物群集構造の遷移

BPA 除去実験の実験系 D における T-RFLP 解析の結果を図 4 に示す。実験系 D では 6 日目から 8 日目にかけて 560 bp 付近のピークの優占度が顕著に高まることが観察され，このピークを示す微生物種が BPA 分解に関与した可能性が示唆された。他方，BPF 除去実験の実験系 B，D では 203 bp のピークの優占度が高まったことから，このピークを示す微生物種が BPF 分解に関与した可能性が考えられた。また，BPA，BPF 除去実験ともに，ヨシを植栽した実験系 D では植栽していない実験系 B に比べて微生物群集の多様性が高いことが観察され，ヨシの存在が微生物群集に影響を及ぼすことが示された。さらに，BPs の除去の進行によっても多様性が高まる傾向が観察され，中間代謝物を資化できる微生物種の増加により微生物多様性が高まった可能性が考えられた。

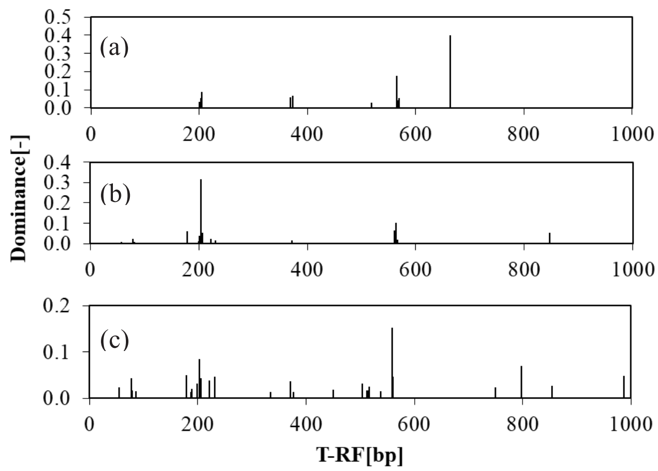

図 4　BPA 除去実験の実験系 D における微生物群集構造の変化 (0 日目(a)，6 日目(b)，8 日目(c))

4.　結論

本研究の結果から，BPF ではヨシ植栽による分解促進効果は見られなかったが，BPA では，ヨシと根圏微生物の存在下において，ヨシが存在しない場合と比べて明確な分解促進が確認され，ヨシの植生が水域における BPA の除去に寄与し得ることが示唆された。また，いずれの BPs の除去実験においても，ヨシが存在する場合に微生物群集の多様性が高まることが確認されたことから，ヨシの植生は汚染物質存在下における微生物生態系の多様性維持という面でも重要な意義を有することが示唆された。

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O3-30

Determination of Chelators in Lake Water using Ultra-Performance Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry

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Keywords: chelating agents or chelators, HILIC amide column, UPLC-Q-TOF-MS, water quality, freshwater

ABSTRACT

The determination of chelators in environmental samples is an analytical challenge due to their structural similarities and presence of low concentrations. We report a rapid and sensitive method for the determination of both the non-biodegradable (ethylenediaminetetraacetic acid, EDTA; N-(2-hydroxyethyl)ethylenediamine-*N*,*N*',*N*'-triaceticacid, EDTA-OH) and biodegradable (ethylenediamine-*N*,*N*'-disuccinic acid, EDDS) chelators as their corresponding Cu^{II}-complexes in the aqueous matrices. An ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry was used. Chromatographic separation was performed using a UPLC HILIC amide column. Sample preparation is easy without pre-treatment steps. Limit of detection was 0.004 $\mu molL^{-1}$ (EDTA), 0.007 $\mu molL^{-1}$ (EDTAOH) and 0.008 $\mu molL^{-1}$ (EDDS). The coefficient of determination (R^2) was 0.999 for the chelators with repeatability of ≤3%. The developed method was successfully applied to the measurement of chelator concentration in lake water samples.

1. INTRODUCTION

Synthetic aminopolycarboxylate chelators are emerging environmental contaminants and extensively used for industrial and domestic purposes worldwide [1]. Chelators are produced in massive quantities every year because of their broad application range. In 2015, global chelators market size was 1026 kilotons. However, continuous exposure of non-biodegradable chelators such as EDTA and its homologs have hazardous environmental effects. Chelators form stable water-soluble complexes with metal ions, which increase metal solubility causing risk to enter the food chain. Although WHO set a tolerable limit of EDTA is 600 μgL^{-1} in drinking water and European Aminopolycarboxylate Committee established no-effect limit of 2.2 mgL^{-1} in an aqueous environment, there were reports of up to 12 mgL^{-1} EDTA in European water bodies [2].

Therefore, sensitive determination technique is essential to check the content of chelators in the open-water bodies. Some methods have been established to determine the chelator and their metal complexes in environmental matrices. However, the detection of metal-chelator complexes is still a challenge due to their structural similarities and presence of low concentrations in environmental samples [3].

The objective of the present study is to develop a rapid, sensitive and precise method for the determination of both non-biodegradable and biodegradable chelators in natural samples using an ultra-performance liquid chromatography quadrupole/time-of-flight mass spectrometry (UPLC-Q-TOF-MS).

2. METHOD

EDTA, EDTA-OH, and EDDS was used in the present study (Fig. 1). The metal-chelator complex solution contained 10^{-2} $molL^{-1}$ Cu^{II} standard, 10^{-3} $molL^{-1}$ chelator standard, 10 $mmolL^{-1}$$(NH_4)_2CO_3$, and acetonitrile / H_2O (70%/30%) as the sample diluents. Real samples from five different lakes, as collected during July to September was used (Fig. 2). Samples were filtered with 0.45 µm filters (Millipore, HA), and stored in polyethylene bottle at 5°C.

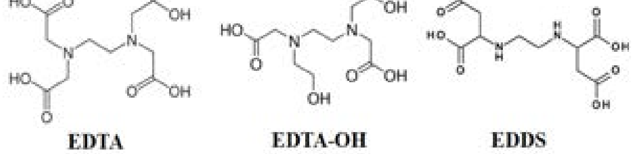

Fig. 1 Structure of chelating agents used in the study

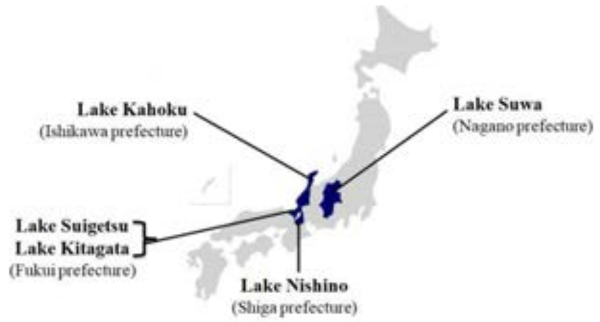

Fig.2 Sampling sites

A UPLC-Q-TOF-MS system (Xevo G2-S QTOF MS, Waters, Milford, USA) was used for the determination of the metal-chelator complexes. Chromatographic separation was achieved using an ACQUITY UPLC BEH amide column (particle size 1.7 µm, 2.1× 150 mm, Waters). A mobile phase consisting of 10 mM $(NH_4)_2CO_3$, and acetonitrile with 0.1 M $(NH_4)_2CO_3$ was used. The gradient condition was as follows: initial, 3% A/97% B; initial -11 min, 3% A/ 97% B to 50% A/ 50%B; 11–13 min, 50%A/ 50% B; 13–13.1 min, 50% A/ 50% B to 3%A/ 97% B. The flow rate of the mobile phase was 0.3 mL min^{-1}, and the injection volume was 10 µL. Acquisition mode was set as negative polarity in resolution analyzer mode. The LC-Q-TOF-MS system was controlled using the MassLynx 4.1 (Waters) software program. The data were generated based on peak intensities, and m/z values were used to identify individual peaks. Peak areas and the signal-to-noise (S/N) ratios were generated using the TargetLynx software as part of MassLynx 4.1.

3. RESULTS

Chelators were determined as their corresponding Cu^{II}-chelator complex. Cu-EDTA, Cu-EDTAOH, and Cu-EDDS samples were analyzed both in positive and negative polarity in electrospray ionization (ESI) mode. Mobile phase composition, pH of the mobile phase, sample diluents, capillary voltage (kV), desolvation gas flow (Lh^{-1}) were optimized. Cu-EDTA (m/z, 352.1), Cu-EDTA-OH (m/z, 338.1) and Cu-EDDS (m/z, 352.1) were detected individually at optimized operating conditions (Fig. 3). Limit of detection (LOD) was calculated based on signal-to-noise ratio of 3. LOD was 0.004, 0.007 and 0.008 $\mu molL^{-1}$ for EDTA, EDTA-OH, and EDDS, respectively. The linearity of the calibration curve was verified by plotting the peak area of the signal versus the amount of chelator added. The coefficient of determination as R^2 was 0.999.

Repeatability of the method was ≤3% as calculated from ten consecutive measurements for a given concentration value. The method validity was checked using five different lake water samples by standard additions (recovery 91.8 to 97.3%). The method was further applied to detect 0.068 $\mu molL^{-1}$ EDTA in lake Nishino located at Shiga prefecture.

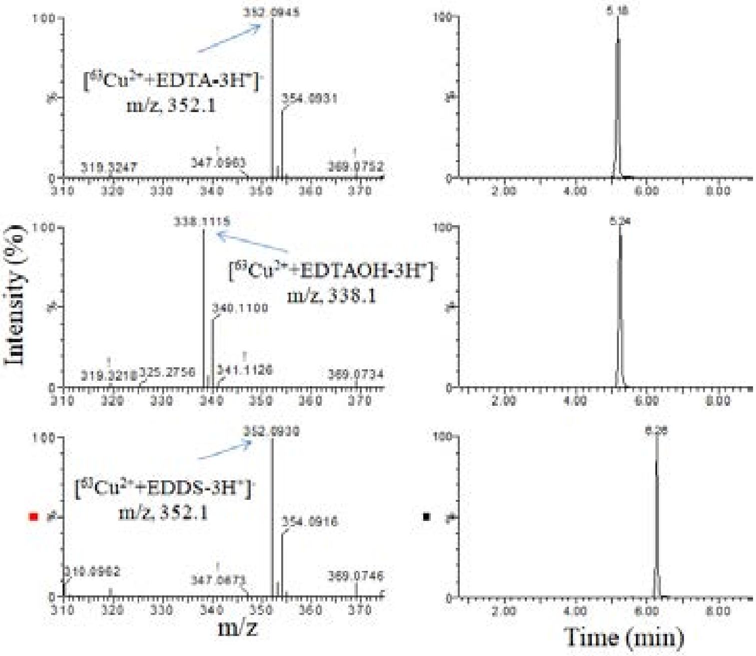

Fig. 3 Mass spectrum and mass chromatogram extracted at optimized operating condition, final concentration of chelator was 2 $\mu molL^{-1}$ in the sample

4. DISCUSSION

The chelators were analyzed in positive and negative polarity in electrospray ionization (ESI) mode by UPLC-Q-TOF-MS. The target peak was detected in both positive and negative mode. However, the signal per noise ratio (S/N ratio) was higher in the negative mode (Fig. 4). The carboxylic group of chelators may facilitate the detection of the anion with high sensitivity. The effect of mobile phase composition and the pH of the mobile phase solution was evaluated for the better sensitivity and satisfactory retention of the analytes. The sensitivities (as S/N ratio) of three Cu-chelator complexes were improved at higher pH (pH 9.0) (Fig. 5). Three mobile phase compositions were selected based on the compatibility with mass spectrometry such as (a) 10mM $(NH_4)_2CO_3$/ acetonitrile + 0.1 M $(NH_4)_2CO_3$ (b) 10 mM CH_3COONH_4/ acetonitrile + 0.1 M CH_3COONH_4 and (c) 10 mM NH_4COOH/ acetonitrile + 0.1 M NH_4COOH. The improved sensitivity of the analytes was achieved when using the composition (a) (Fig. 6). The satisfactory retention was also observed using this mobile phase composition. The desolvation gas flow (Lh^{-1}) and the capillary voltage (kV) were optimized during the measurement by UPLC-Q-TOF-MS.

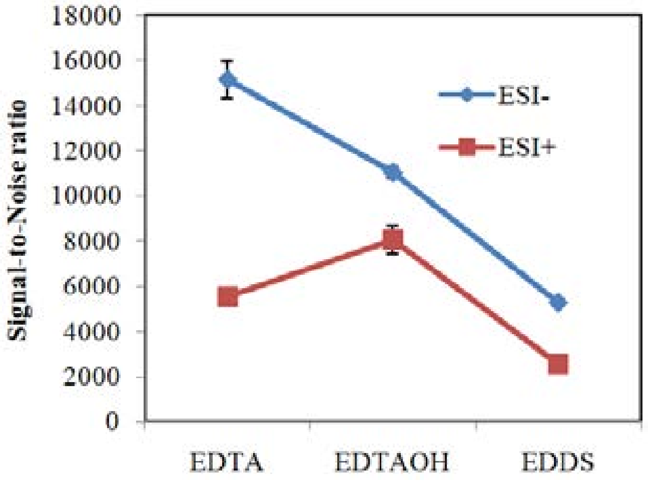

Fig. 4 Selection of polarity (positive/negative) in ESI mode for the determination of Cu-chelator complexes, final concentration of chelator was 10 µmolL^{-1}

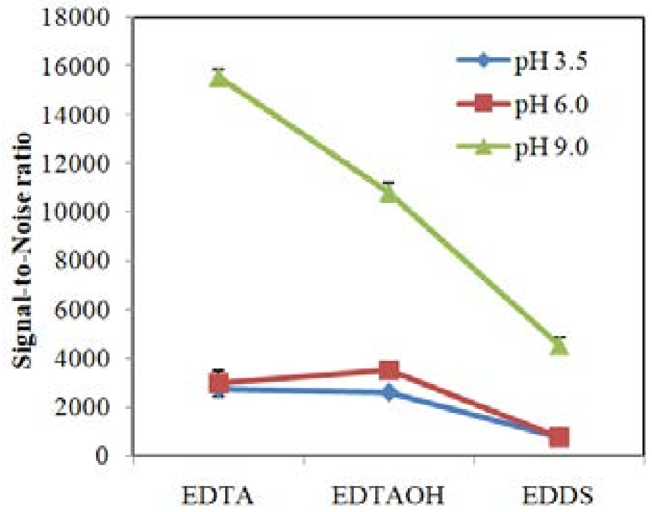

Fig. 5 Effects of mobile phase pH on sensitivity of Cu-chelator complexes, final concentration of chelator was 10 µmolL^{-1}

Five lake water (Fig. 2) samples were analyzed among which Lake Nishino of Shiga prefecture has shown the detection of 0.068±0.08 µmol L^{-1} EDTA content, whereas other found below the detectable limit. It would better be noted that EDTA might hamper the biological process at >1 mgL^{-1} concentrations [4]. The proposed technique was fast, sensitive and useful for the determination of chelating agents from natural samples.

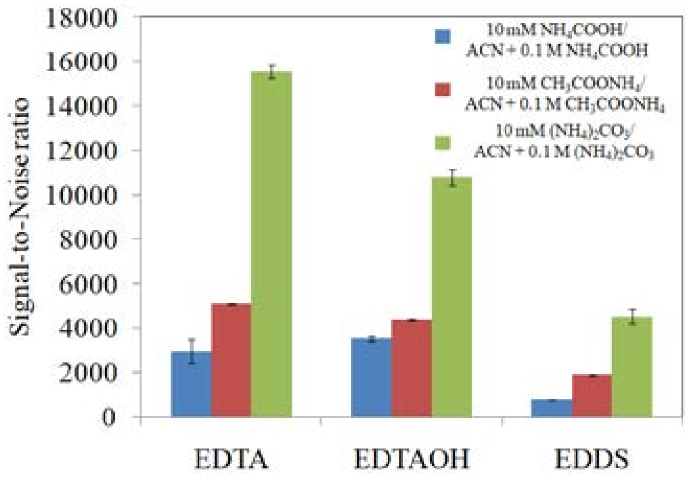

Fig. 6 Effects of mobile phase composition on the sensitivity of Cu-chelator complex, final concentration of chelator was 10 µmolL^{-1}, ACN indicates acetonitrile

5. CONCLUSION

The strengths of the proposed method include rapid determination, fast separation, and high sensitivity and resolution, and no pretreatment step to enrich the target species.The method was also successfully applied to the determination of Cu-EDTA complexes inlake water samples.

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O3-31

Characteristics of phosphorus sorption-desorption behaviour and effects of salinity, pH and temperature on phosphorus sorption in sediments of a largest brackish water lagoon, Chilika, South Asia – A case study

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Abstract

The lagoon, Chilika positioned as one of the largest brackish water ecosystems and designed as a Ramsar site in South Asian continent. Phosphorus sorption and desorption process can able to predict the status of phosphorus as the source or sink for coastal lagoon ecosystems. A very few studies on this aspect has been standardized for coastal lagoon in the south Asian continent. The sorption-desorption processes standardize in the laboratory maintaining the same environmental condition of the lagoon, Chilika. The effects of major water quality parameters like salinity, pH and temperature on phosphorus sorption on sediments were studied for the outer channel (OC), northern sector (NS) and southern sector (SS) in the lagoon, Chilika. The lagoon sediment for phosphorus sorption could be better assigned by the modified Langmuir model than by the modified Freundlich model. Effect of salinity, pH, and temperature on phosphorus sorption could be better understood by a binomial equation. The rate of phosphorus sorption increases with increasing salinity, pH, and temperature at low ranges. Whereas it decreases in excess of threshold values. The maximum phosphorus sorption capacity (Q_{max}) was more for NS sediments (258 mg/kg) compared with OC (219 mg/kg) and SS sediments (237 mg/kg) ($p < 0.05$). The percentage of phosphorus desorption (P_{des}) in the NS sediments (8.5-64.5%) was much lower than those in OC (29.7-126.9%) and SS sediments (21-109.5%). This study can able to help wetland manager to control eutrophication status of the lagoon by immediate effect of increasing P sorption through freshwater restoration.

Key words: Phosphorus; sorption; desorption; isotherm models; brackish water lagoon

O3-32

Characteristic of the photochemical release of phosphate from resuspended sediments under solar irradiation

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Keywords: sediment, resuspension, phosphorus, photochemical, vertical distribution

ABSTRACT

In previous studies, resuspended sediments that were exposed to simulated solar irradiation could release dissolved phosphate (PO_4^{3-}). However, the mechanisms of phosphate release remain unclear. In this research, a battery of experiments was performed to reveal the mechanisms of the photochemical release of phosphate from resuspended sediments of a shallow eutrophic lake under solar irradiation. The results show that the PO_4^{3-} released in resuspended sediments was significantly higher than that in the dark control or in water alone after treatment with solar irradiation for 6 h. The results of sequential chemical extractions showed that the concentrations of labile organic, moderately labile organic and residual organic phosphorus decreased in the resuspended sediment after 6 h of solar irradiation; of these, moderately labile organic phosphorus was the greatest contributor to the release of dissolved phosphate in resuspended sediment. Orthophosphate, phosphate monoesters, phosphate diesters and pyrophosphate were detected with ^{31}P-NMR. It is worth mentioning that the diester- P and pyro-P species disappeared after 6 h of irradiation. All of these results suggest that photochemical processes may lead to PO_4^{3-} release from sediment in aquatic environments. Moreover, the content of photochemical release of PO_4^{3-} increased with the increasing of suspension concentration within a certain concentration range and the photochemical release of PO_4^{3-} has significant spectral sensitivity.

1. INTRODUCTION

A recent study demonstrated that dissolved nutrients could be released when resuspended marine and estuarine sediments were irradiated by sunlight [1,2,7]. In a suspension constituted with autoclaved sediment (< 30 μm) and filtered seawater, more dissolved PO_4^{3-} was released under solar irradiation than in dark conditions. Because biological effects had been eliminated, the increase in PO_4^{3-} in the suspension was attributed to the photochemical decomposition of organic phosphorus from sediments [8]. These findings suggested that photo catalysis had a significant impact on organic phosphorus production into the surface waters, leading to the increase of the concentration of available phosphorus [9]. These earlier studies have found that photochemical processes play an important role in nutrient recycling in aquatic environments, but there is scant research of environmental controls and mechanisms of the phosphate released from resuspended sediments under solar irradiation.

Here, we undertook a detailed study of PO_4^{3-} release from resuspended sediments of a shallow eutrophic lake, under simulated solar irradiation. Sequential chemical extractions and ^{31}P-NMR were used to investigate variations in the phosphorus speciation during sediment resuspension under solar irradiation. Environmental parameters, such as the initial concentration of the resuspended sediment and the radiation spectrum were considered. The results will be useful to understanding the phosphorus cycle in aquatic environment.

2. METHOD

The water and sediment used in the experiment were collected from Lake Nan in Wuhan, China. Sediment samples were mixed,and autoclaved at 115–120 °C for 30 min. The mixed lake water was stored at 4 °C after being filtered with a 0.2 μm polysulfone filter (Supor-200, PALL) to remove microbes and suspended particulate. The fractionate and speciation change in organic phosphorus before and after solar irradiation was determined by sequential extraction procedure and ^{31}P-NMR [3]. Overall, the concentration of dissolved phosphate was determined using the molybdenum blue method.

3. RESULT

3.1. Release of PO_4^{3-} during sediment resuspension

As shown in Fig. 1a and b, the initial concentration of PO_4^{3-} in the filtered water and filtered autoclaved sediment resuspension was 0.18 and 0.30 mg/L, respectively. After solar irradiation for 6 h, there was no significant difference in the PO_4^{3-} concentration in the filtered water. However, the PO_4^{3-} concentration increased from 0.30 to 0.32 mg/L in the filtered autoclaved sediment resuspension after 6 h of solar irradiation. Most notably, the concentration of PO_4^{3-} increased from 0.31 to 0.41 mg/L in the suspension after solar irradiation for 6 h (Fig. 1c), which suggested that the resuspended sediments that were exposed to simulated solar irradiation could quickly release PO_4^{3-}.

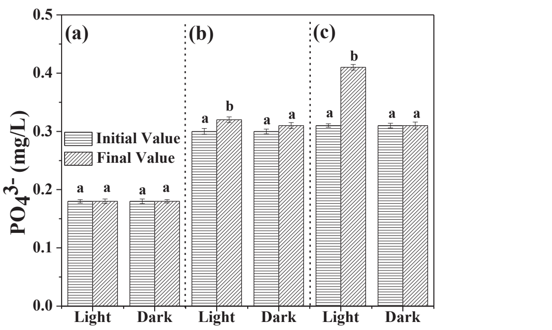

Fig. 1 The release of PO_4^{3-} in (a) filtered water, (b) filtered autoclaved sediment resuspension and (c) autoclaved sediment resuspension under solar irradiation.

3.2. Fractionation of phosphorus during sediment resuspension under solar irradiation

In this work, the organic phosphorus in the autoclaved resuspended sediments were divided into the labile organic (LOP), moderately labile organic (MLOP) and non-labile organic phosphorus (NLOP) groups, as in Lü's report [5, 6]. As shown in Table 1, the concentration of the different components of organic phosphorus was significantly lower after solar irradiation, comparedwith before the solar irradiation. The mean concentrations of LOP, MLOP and NLOP decreased from29.29 ± 2.93, 185.66± 3.38 and 42.02 ± 4.22mg/kg to 12.79± 2.53, 105.71 ± 4.24 and 23.47±2.21mg/kg, respectively. The results from ^{31}P-NMR indicated that the resuspended sediments were composed predominantly orthophosphate (ortho-P), phosphate monoesters (mono-P), phosphate diesters (diester-P) and pyrophosphate (pyro-P) (Fig.2). In the process of solar irradiation, the peaks of the diester-P and pyro-P disappeared .Meanwhile, the peak formono-P also changed after solar irradiation, indicating that the constituents of the mono-P changed.

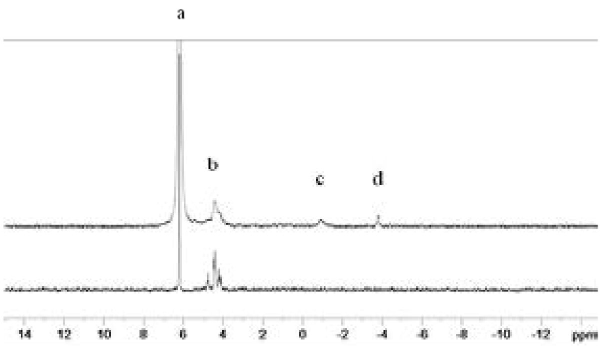

Fig. 2 ^{31}P-NMR spectra of NaOH–EDTA extracts of the resuspended sediments before and after solar irradiation for 6 h: (a) Ortho-P; (b) Monoester-P; (c) Diesters-P; (d) Pyro-P.

Table1 Different organic phosphorus concentrations in resuspension sediments

Classification of OP	Value (mg/kg)		Transformation rate (%)
	Before irradiation	After irradiation	
LOP	29.29 ± 2.93	12.79 ± 2.53	56.33 ± 1.12
MLOP	185.66 ±3.38	105.71± 4.24	43.06 ± 1.12
LOP	42.02 ± 4.22	23.47 ± 2.21	44.15 ± 1.12

3.3. Effects of environmental parameters on the photorelease of PO_4^{3-} during sediment resuspension

The environmental parameters are important factors that influence the photochemical release of PO_4^{3-} during sediment resuspension. As shown in Fig.3a, the initial concentrations of the resuspended sediment in the resuspension prepared by adding 1, 2 and 3 g of autoclaved sediment was 33, 73 and 101 mg/L, respectively. And the initial concentration of PO_4^{3-} in the suspensions was 0.28, 0.31 and 0.32 mg/L, respectively. The initial concentration of PO_4^{3-} increased with increasing amounts of resuspended sediment. After 6 h of solar irradiation, the concentration of PO_4^{3-} increased to 0.34, 0.41 and 0.43 mg/L, and the release of PO_4^{3-} increased by 0.06, 0.10 and 0.11 mg/L in the samples with the resuspended sediment concentrations of 33, 73 and 101 mg/L, respectively.

PO_4^{3-} released from resuspended sediment under solar and visible light irradiation, and a 420 nm filter was used to remove the light that has wavelengths shorter than 420 nm. As shown in Fig. 2b, the concentration of PO_4^{3-} in the experiments with solar and visible light irradiation increased to 0.44 and 0.33 mg/L, respectively.

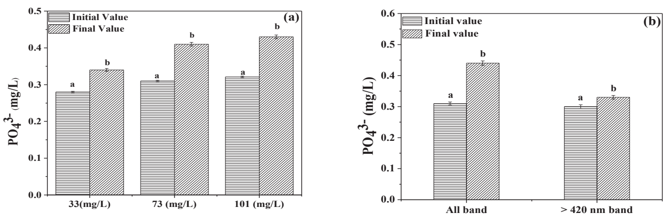

Fig. 3 Effect of environmental parameters on the PO_4^{3-} photo-released from resuspended sediment under solar irradiation: (a) initial concentration of resuspended sediment and (b) irradiation spectrum

4. DISCUSSION

Dark control was set up in this experiment, as shown in Fig.1. Because the biological effects were removed,the increase in dissolved PO_4^{3-} concentration should be attributed to the photodegradation of organic phosphorus. Compared to the amount of PO_4^{3-} that resulted from the dissolved organic phosphorus, the photodegradation of particulate organic phosphorus to PO_4^{3-} played an important role in phosphorus release.

The concentration of resuspended sediment could influence the initial concentration of phosphate and organic phosphorus in the water environment. In addition, as reported by Mayer, high concentrations of resuspended sediment could block the transmission of light in the water [8]. However, as shown in Fig.3a, when the resuspended sediment concentration increased to 101 mg/L, the amount of PO_4^{3-} that was released decreased. This result is mainly because the distribution of dense particles blocked the transmission of light in the suspension with a high sediment concentration, inhibiting the photolytic degradation of organic phosphorus during the sediment resuspension.

The radiation spectrum significantly affects the number of free radicals that are generated, which will further influence the photodegradation of organic matter in the natural aquatic environment [4]. The Fe^{3+} ion has also been shown to be a photoreactive species, in terms of •OH generation irradiation with visible light [10,11]. In this experiment, The amount of PO_4^{3-} released from resuspended sediment under solar irradiation was higher than that of visible light treatment, which suggested that PO_4^{3-} released from organic phosphorus photodegradation during sediment resuspension was highly spectrum-sensitive.

5. CONCLUSION

This study revealed the mechanism of PO_4^{3-} release from resuspended sediments under solar irradiation. The amount of PO_4^{3-} that was released dramatically increased when the sediment resuspension occurred under solar irradiation, and the initial concentration of the resuspended sediment and radiation spectrum affected this process significantly. ^{31}P- NMR and the sequential extraction of organic phosphorus clearly demonstrated that the organic phosphorus component changed when the resuspended sedimentswere exposed solar irradiation. Together, these results benefit our understanding of the phosphorus cycle in the process of shallow lake eutrophication. Continued investigation is necessary to understand the transformation process of particulate phosphorus to phosphate under solar irradiation and to fully understand the fate and transport of sediment phosphorus

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O3-33

Spatio-Temporal Variability of Water Quality in a Large Shallow lake In Southeast Asia: Tonle Sap Lake, Cambodia

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Keywords: Tonle Sap Lake, water quality, spatio-temporal variability

ABSTRACT

Tonle Sap Lake (TSL) in lower Mekong River Basin and also is the largest freshwater lake in Southeast Asia at the end of the wet season. The water quality and decline in biological resources have been raised regarding the degradation of water quality in Tonle Sap Lake. The study aimed to study variability in water quality in the large shallow lake scale at temporal and spatial scale; and to better understand the state of water quality in this largest Southeast Asia shallow lake. The presented study was based on surface water quality collected from cross-section of Tonle Sap Lake covering a maximum of 37 sampling stations during December 2016 and June 2017 corresponding to receding and rising stage of lake volume, respectively. Variation of water depth was high between December and June (on average 2.7 m in June and 4.7 m in December) and caused high variability for TSS (170 mg/l in June and 7 mg/l in December). However, low variability of rising and receding stage had been detected for DO. Based on information obtained from this study, it provided the information regarding to the spatio-temporal water quality in various layers to understand the state of water quality in TSL. Additionally, the integration of process-based information with interpolation maps could support the further study of prior sampling points or monitoring program.

1. INTRODUCTION

The Tonle Sap Lake (TSL) and its floodplains contain the largest continuous areas of natural wetlands habitats remaining in the Mekong system while being the largest permanent freshwater body in Southeast Asia. During the dry season, November to April, the lake size is about 120 km long and 35 km wide, with an area of about 2500 km^2 and flow to Mekong River from the northwest part. In the wet season, from May to October, flooding and the associated water level increase in the Mekong River causes the Tonle Sap River to change flow direction and flow towards the northwest (upstream) into Tonle Sap Lake [1]. During the flood phases, the lake receives a large amount of water volume from Mekong River through reversal flow and enlarges to about 250 km long and 100 km wide, with an area of about 17,500 km^2 [2]. Some concerns have been raised regarding the degradation of water quality in Tonle Sap Lake due to multiple pressures ranging from human activities in floating houses in the lake to agricultural activities in watershed [3]. Investigations into the variations in water quality should be based on monitoring over a sufficient spatial extent to the whole lake. However, due to the variation of the size during dry and wet season of the Tonle Sap Lake, spatial and temporal variations studies in the water quality of Tonle Sap Lake remain limited.

The presented study was based on surface water quality collected in December 2016 and June 2017 in Tonle Sap Lake corresponding to receding and rising stage of lake volume, respectively. The study aimed to study the variability in water quality in the large shallow lake scale at temporal and spatial scale.

2. METHOD

The data collection comprised of 37 sampling stations and two sampling campaigns in December 2016 and June 2017 corresponding the beginning of the receding stage and beginning of the rising stage of the lake volume. The exact sampling locations were fixed by using Global Positioning System (GPS). All the sampling locations (Fig. 1) have been projected on cross-section lines (CS) passing through the entire lake. Surface water layer and bottom water layer (0.5 m above the bed) were collected for this study from the lake, as the depth of water is less 3m and 7m in the dry and wet season, respectively. Temporal variations of different parameters had been assessed using descriptive statistic. The ordinary kriging (OK) had been performed to map selected water quality since it could provide outperforms than other spatial interpolation methods in Tonle Sap Lake for both layers [4]. Targeted parameters are total depth, Electrical conductivity, water temperature, oxidation-reduction potential, pH, dissolved oxygen, Turbidity, Total suspended solid. All water quality parameters (except TSS) were conducted in-situ measurement using YSI EXO Water Quality Sondes. The sensors had been calibrated regularly (before conducting the field survey) to ensure accuracy and response times. TSS had been conducted analysis in laboratory adapted from American Public Health Association (APHA).

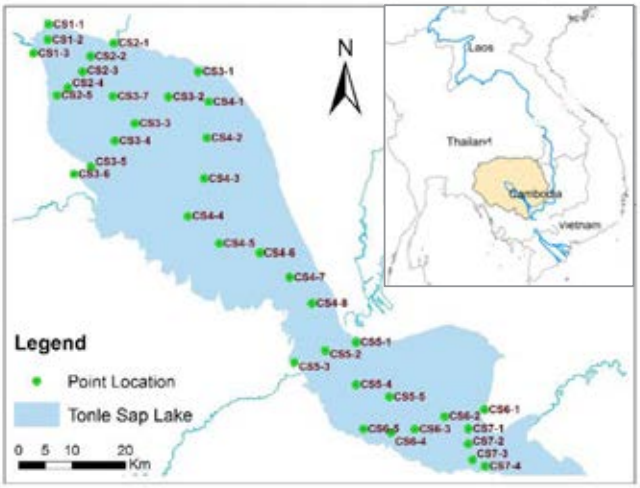

Figure 1: Sampling locations in Tonle Sap Lake

3. RESULTS AND DISCUSSION

Temporal water quality

The descriptive statistic was applied to the dataset and showed in Table 1. The result enabled us to compare water quality parameters between two separated stages.

Table 1: Descriptive statistic of the selected water quality parameters during December, 2016 and June, 2017 in-situ measurement campaigns

		Surface Layer							Bottom layer						
	Parameters	Min	Max	Average	S.D	C.V	Skewness	Kurtosis	Min	Max	Average	S.D	C.V	Skewness	Kurtosis
Jun-17	Total Depth (m)	1.60	5.53	2.78	0.64	0.23	1.98	9.02	1.60	5.53	2.78	0.64	0.23	1.98	9.02
	Temp (°C)	29.30	35.70	31.98	1.36	0.04	0.56	0.28	28.90	32.30	30.09	0.67	0.02	0.88	1.81
	EC (µS/cm)	51.40	248.0	154.75	37.00	0.24	-0.49	1.68	40.40	211.00	142.28	31.83	0.22	-1.02	2.29
	pH	6.66	8.94	7.84	0.64	0.08	0.17	-0.97	6.66	8.55	7.63	0.46	0.06	-0.29	-0.59
	ORP (mV)	138.0	250.0	186.6	29.6	0.16	0.18	-0.75	19.0	240.0	188.8	42.0	0.22	-2.94	10.12
	DO (mg/L)	4.11	10.93	7.08	1.57	0.22	0.11	0.03	3.38	7.67	6.28	0.94	0.15	-1.66	2.93
	TSS (m/l)	4.50	405.0	169.50	103.22	0.61	0.42	-0.23	10.00	716.0	160.6	208.9	1.30	1.68	1.76
		Surface Layer							Bottom layer						
Dec-16	Total Depth (m)	3.55	8.78	4.75	0.86	0.18	2.93	13.37	3.55	8.78	4.75	0.86	0.18	2.93	13.37
	Temp (°C)	25.70	29.40	27.15	0.87	0.03	0.52	0.13	25.60	28.70	26.81	0.90	0.03	0.68	-0.37
	EC (µS/cm)	47.90	140.70	101.73	24.16	0.24	-0.70	-0.27	25.80	143.70	100.60	27.43	0.27	-1.06	0.79
	pH	6.82	8.34	7.55	0.40	0.05	-0.17	-0.96	6.51	8.10	7.45	0.43	0.06	-0.45	-0.88
	ORP (mV)	137.0	472.0	238.7	108.7	0.46	1.27	0.01	19.2	462.0	228.4	112.1	0.49	1.01	-0.04
	DO (mg/L)	1.53	8.70	6.58	2.03	0.31	-1.39	0.87	2.47	8.27	6.62	1.56	0.24	-1.12	0.46
	Turb (NTU)	2.60	36.00	14.67	8.27	0.56	0.61	-0.14	3.25	53.40	17.52	10.15	0.58	1.28	2.97
	TSS (m/l)	3.31	11.50	7.91	2.04	0.26	-0.03	-0.42	4.50	24.0	9.65	3.66	0.38	1.63	5.26

Note: Min: minimum, Max: maximum, S.D: standard deviation, C.V: coefficient variation

The table showed the high variation of water depth in December and June. At the beginning of the receding stage, water depth ranged from 3.5 to 8.7 meters in the lake and result the lake surface was larger than June (water depth ranged from 1.6 meters to 5.5 meters). In general, the concentrations of most water quality parameters shall differ between seasons or lake volume. In June, beginning of rainy season, water levels were low which resulted in an accumulation of TSS in the lake and reached a high level. These were confirmed by both water layers, the surface layer (4-450 mg/l, on average 170 mg/l) and the bottom layer (10-710 mg/l, on average 160 mg/l). The TSS concentration is diluted by monsoonal rainfall events after the beginning of rainy season, inflow from the Mekong River and high lake volume.

After the peak water depth in between October and November, water in Tonle Sap Lake starts to fall and December is the beginning of the receding stage. However, the water level was still in the high level during the study period. Water depths ranged between 3.5 meters to 8.7 meters and on average was 4.7 meters. The turbidity and TSS concentration in this stage were diluted by lake volume; Turbidity was in between 2.6 to 36 NTU (average 14 NTU) for the surface layer and in between 3.2 to 53 NTU (average 17 NTU) for the bottom layer. TSS concentration also showed the decrease trend during these periods as on average was 7.9 mg/l for surface layer and 9.6 mg/l for the bottom layer. Turbidity and TSS established a good agreement that this shallow lake clarification follows the variability of the lake size and volume.

Regarding DO, the low variability of this rising and receding stage has been detected. In June 2017, the average DO was 7 mg/l and 6.2 mg/l for surface and bottom layer. In December 2016, DO was 6.5 mg/l and 6.6 mg/l for surface and bottom layer. Between DO and pH in both observed months showed good positive correlation and could indicate the production of dissolved oxygen by photosynthetic activity.

Spatial Variability of Water Quality

TSS, DO and EC was selected for spatial variability study based on high variability among the sampling stations and importance of each parameter. A coefficient variation (CV) was used to compare variability between different sampling stations. TSS is one of the indicators for lake clarification, DO is an essential factor for aquatic life, while EC of lake water is an estimator of the amount of total dissolved salts. The distribution maps of selected water quality parameters had been constructed to visualize the spatial variability in both water surface layer and bottom layer during rising and receding stage of the lake volume. The ordinary kriging (OK) had been performed to map selected water quality in Tonle Sap Lake for observed months and both layers [4] (Figure 2).

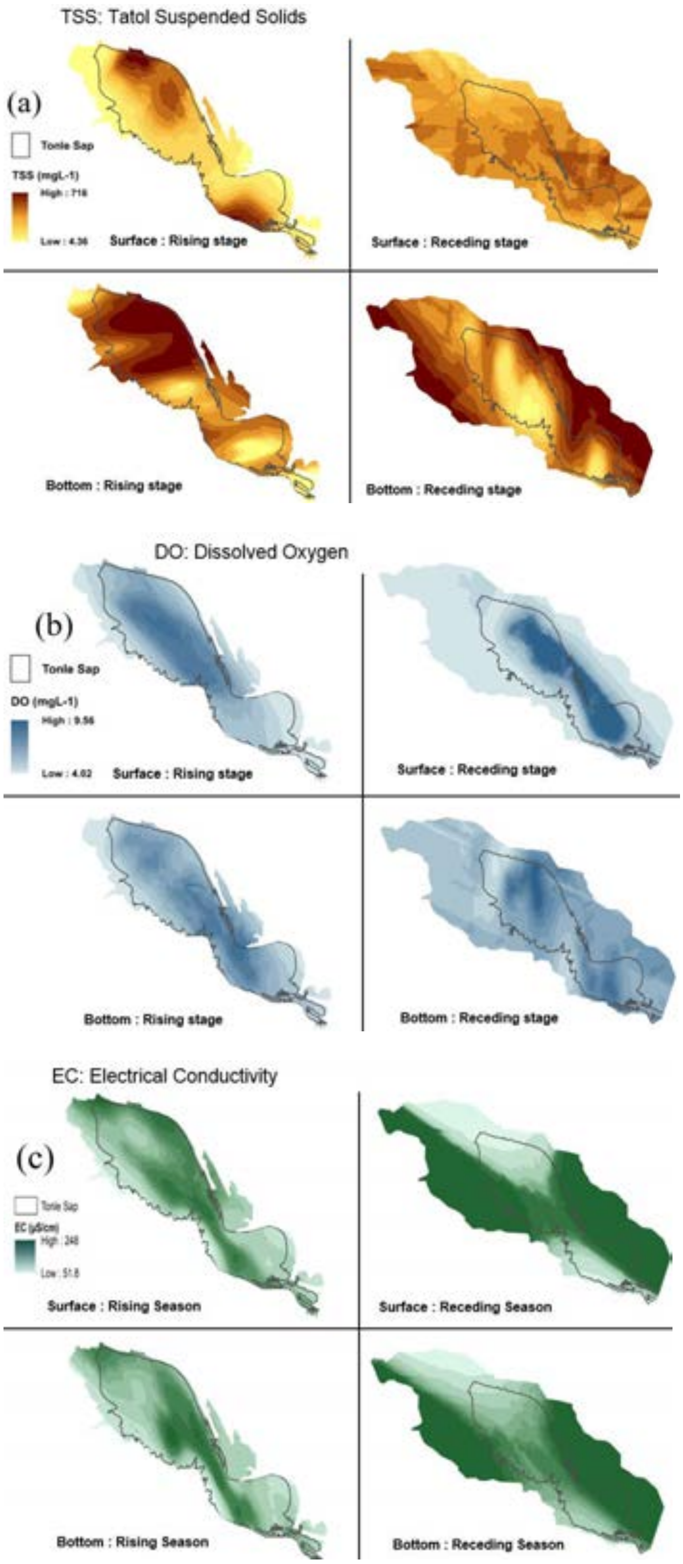

Figure 2: Distribution maps of selected water quality in Tonle Sap Lake and its floodplain, (a) TSS, (b) DO and (c) EC. Top left: surface layer in rising stage, Bottom left: bottom layer in rising stage, Top right: surface layer in receding stage, Bottom right: bottom layer in receding stage.

In June, TSS concentration was high in upper part of the lake in both surface and the bottom layer (160-400 mg/l). Another concentrated area of TSS was in lower part (180-450 mg/l) for the surface layer. However, bottom layer concentrations of TSS were lower in this area (15-65 mg/l). In receding stage, TSS concentration was low, in particular, in the middle of the lake (5-25 mg/l). The contrast of TSS, DO was good in the middle of the lake in these months and bother layer (5-9 mg/l) because of dilution from the lake volume. While near the lake boundary, DO was lower (4-6 mg/l) due to agricultural activities around the lake and floating villages in that area. EC provided different spatial variability in June and December, but good agreement in surface and bottom layer. In June, high EC (150-200 µS/cm) occurred in the middle of the lake but in December, the middle of the lake, EC was lower (20-150 µS/cm).

4. CONCLUSION

In this study, descriptive statistic and geospatial interpolation method had been used for spatio-temporal study of water quality collected in December 2016 (receding stage) and June 2017 (rising stage) in Tonle Sap Lake. Based on results obtained from this study, it could provide information regarding the spatio-temporal water quality in various layers to understand the state of water quality. Additionally, the integration of process-based information with interpolation maps could support the further study of prior sampling points or monitoring program. The targeted parameters can be used in the future study to establish correlations among parameters.

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O3-34

Status of biological water quality of main rivers connected to Tonle Sap lake, Cambodia

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Keywords: Biological water quality, lake, river, wastewater, environmental water

ABSTRACT

Water sources in the Cambodia are being contaminated by the untreated wastewater discharge from the city; however, the biological water quality data is scarce in the region. We investigate the presence of *Escherichia coli* (*E. coli*), coliform and its gene (*uidA*) in Mekong river, Tonle Sap river and Tonle Sap lake since this environmental water was affected by domestic wastewater. Concentration of *E. coli* and coliform in Tonle Sap river were higher than those in Tonle Sap lake and Mekong river. *E. coli* concentration in Tonle Sap river is about 10 times higher than in Tonle Sap lake and about 100 times higher than in Mekong river. The *uidA* gene was identified in Tonle Sap river almost every month from March to December 2017 while *uidA* was identified in Mekong river only from March to May 2017. The results from this study demonstrated that the main water sources in the country such as Tonle Sap lake, Tonle Sap river and Mekong river are under the pressure of fecal contaminated source. The untreated wastewater from the city, which may contain high concentration of virulent microorganisms, was discharged into the water body in daily basis. The construction of wastewater treatment plant in the country is necessary for removal of unwanted substances or microbes for improving the water source quality and for sustainable development.

1. INTRODUCTION

Cambodia economy gradually rose year by year with the average of 7.6% growth rate from 1994 to 2015 (http://www.worldbank.org/en/country/cambodia/overview). The infrastructure in the country is much improving recently, particularly in Phnom Penh capital city. Cambodia economy largely depends on the natural resources such as agriculture and fisheries production.

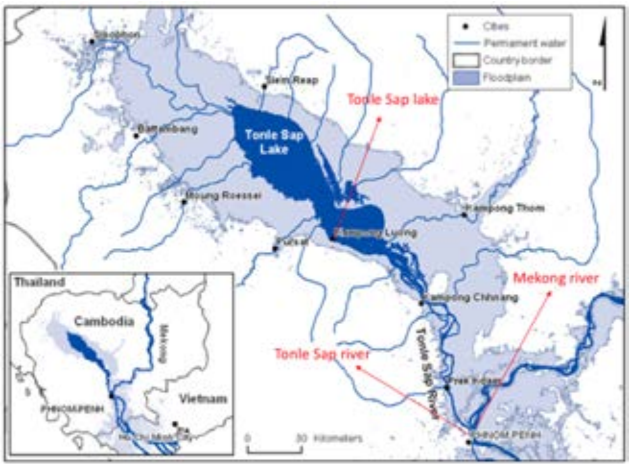

Fig.1 Sampling point of Mekong river and Tonle Sap river and Tonle Sap lake.

Tonle Sap lake, the largest fresh water body in Southeast Asia, offer uncountable benefit for Cambodia and play an important role in flood regulation in the region[1,2]. The water cycle in this lake is exceptionally unique. The water usually flows from Mekong river into the lake in rainy season (from December to April) and flow from the lake to Mekong river via Tonle Sap river in dry season (from May to October). The lake and rivers offer a ton of fish, snake, shrimp, and other aqua products for supporting the food demand[3,4].

The connection between Mekong river and Tonle Sap river is located at the capital city of Phnom Penh, Cambodia. Since, there is no wastewater treatment plant in Phnom Penh, the untreated wastewater was discharge into water source such as Tonle Sap river, Mekong river, Bassac river and wetland[5]. Even though those water sources provide uncountable advantages for human, they were reported under threat due to the inflow of not only the domestic wastewater but also industrial, agricultural, and floating villages waste. As an estimation, more than 234 tons of feces was discharged into water source and among of those waste, 77 tons was discharge into the lake body in daily basis. There is a plenty of water in the country, but 66% of people could not access safe water and 65% of water pollution derived from domestic wastewater[6,7]. Those wastewaters may highly have contributed to lake and river water quality and driven the negative impact on the human; especially floating villager, since they use the lake water for their daily life activities[2,7,8]. As a reported consequence, the numbers and the species of diversity in water source such as fish, snake, as well as wildlife, were declined gradually[1,9,10]. Hundred cases of typhoid and pediatric bloodstream infections caused by pathogenic bacteria were determined in the hospital[4,11]. The mortality rate of children under five years in Cambodia is high in Southeast Asia. Cambodia has loss US$448 million per year for health cost due to poor sanitation[6,12].

Even though many cases of waterborne diseases were reported, the biological water quality data in the country is very scarce in the region and people are still exposed to health risk. Understanding the status of biological water quality of water source in the country in necessary. Our study's aim is to determine the presence and concentration of *Escherichia coli* (*E. coli*), coliforms and *uidA* gene in Tonle Sap river, Mekong river and Tonle Sap lake as affected by anthropogenic pollutants.

2. METHOD

2.1 Water sample

Water sample was taken from a Kampong Loung floating village, Tonle Sap lake (12.57383, 104.2082) in March 2017 and samples were taken from Tonle Sap river (11.586765, 104.920232) and Mekong river (11.590092, 104.938058) from March to December 2017 for the analysis (Fig. 1). A part of samples was used for plating and other water samples were filtrated through 0.45 µm mixed cellulose ester membrane (A045A025A, ADVANTEC). The samples were store in 70% ethanol for the analysis.

2.2 Culture based method

To count the number of *E. coli* and coliforms in water samples, Chromocult® Coliform Agar (Merck KGaA, Germany) was used. *E. coli* produces a purple colony, coliform produces pink colony and other bacteria produce white colony on the Chromocult plate.

2.3 Gene based method

The membrane after sample filtration in 70% ethanol was transferred into glass bead (ø 0.1 mm and ø 0.5 mm) tube. The suspension in ethanol phase was centrifuge at 8,000 g for 10 min and the pellet was resuspended with 200 µL pure water. The resuspension was transferred to the glass bead tube contained the membrane. The further DNA extraction process was described in the literature[13]. DNA template was precipitated in isopropanol containing 1 µL of glycogen at - 20ºC for 30 min. After centrifugation at 15,000 g for 15 min, the pellet was washed with 70% ethanol. After drying, the DNA templates were finally dissolved with 30 µL TE buffer and measured their concentration and purity by spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific). The DNA templates were store at - 20ºC for further analysis. The oligonucleotides primer (*uidA* gene, 162 bp) was constructed following the reported paper[14]. To identify uidA gene presented in *E. coli*, the DNA templates were subjected for PCR amplification as follows: 95°C for 53 min of initial denaturation step, 35 amplification cycles (95°C for 30 s of denaturation, 58°C for 30 s of annealing, and 72°C for 60 s of elongation), and 72ºC for 10 min of final extension step. The PCR products were loaded in gel electrophorese and the band of target gene were confirmed under UV light.

3. RESULTS

E. coli and coliforms bacteria have been used as indicators of water quality and fecal contamination in environmental water. Our analysis in Fig. 2 showed that the concentration of *E. coli* and coliforms were found the highest in Tonle Sap river while the lowest were found in Mekong river. Coliform concentration is above 10 times higher than *E. coli* concentration in Tonle Sap lake and Tonle Sap river. However, coliform concentration is about 100 times higher than *E. coli* in Mekong river.

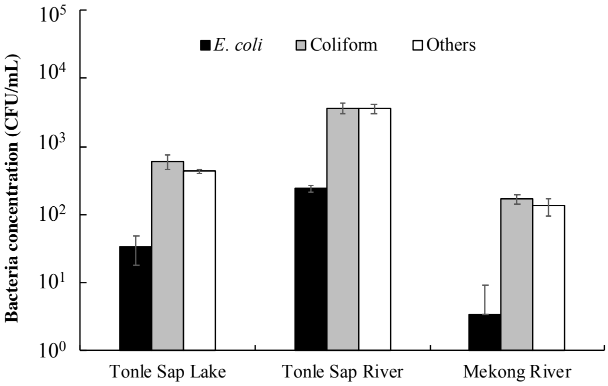

Fig.2 Concentration of *E. coli* and coliforms in Tonle Sap lake, Tonle Sap river and Mekong river.

By using one of the specific gene (*uidA*) in *E. coli*, we determined its existence in Tonle Sap river and Mekong river from March to December 2017 (Fig. 3). We detected *uidA* gene almost in all samples that were taken from different month in dry season of Tonle Sap river, except rainy season from October to November 2017. However, *uidA* gene was detected in some of Mekong river samples in March, April and May 2017. In the samples from following month in rainy season, *uidA* gene was not detected.

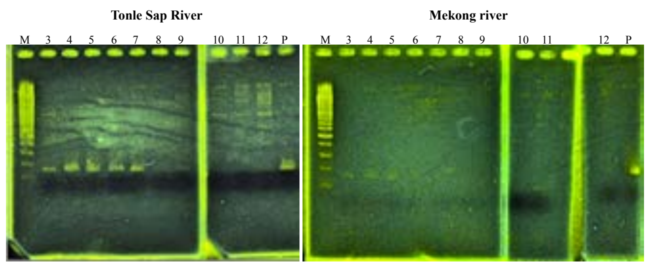

Fig. 3 Detection of *uidA* gene in Tonle Sap river and Mekong river from March to December 2017. Number represented the sampling for each month from March to December, 2017. M: marker, 1: January, 2: February, 3: March, 4: April, 5-May, 6-June, 7-July, 8-August, 9-September, 10-October, 11: November, 12: December, P is positive control.

4. DISCUSSION

Balancing between country development and environmental status is necessary for sustainable development. For understanding the basic biological water quality, detection of indicator of pathogenic bacteria concentration such as *E. coli*, coliforms and its gene in environmental water is important. The presence and the consistence of *E. coli*, coliforms and *uidA* gene in Tonle Sap lake, Tonle Sap river and Mekong river illustrated that the water source was recently or simultaneously polluted from the fecal wastewater source derived from humans. However, *E. coli* and coliforms concentration was lower in Mekong river than Tonle Sap river and Tonle Sap lake. It's reasonable since the water volume of Mekong river is larger than Tonle Sap river and less contaminated from capital city. The polluted water may be threatening the diversity in water source and human health, especially the floating villagers since they used the lake water for their daily life activities.

The results investigated in this study was clearly showed that the river water in Phnom Penh city and also Tonle Sap lake was continuously contaminated from human waste. High concentration of *E. coli* and coliform found in those water sources may reflect the health and environmental vulnerability in a developing country such as Cambodia. Wastewater treatment plant is needed for a speedy developing city such as Phnom Penh and the development of an appropriate septic tank is also necessary for floating villagers.

5. CONCLUSION

High concentration of *E. coli* and coliform were found in Tonle Sap river and Tonle Sap lake while their abundance was low in Mekong river. The *uidA* gene was also detected in Tonle Sap river and Mekong river especially in dry season.

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O3-35

Survival of *Escherichia coli* K12 and Detection of Antibiotic-resistant Bacteria in Tonle Sap, Mekong and Basac Rivers

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Keywords: Tonle Sap River, Mekong River, Basac River, *E. coli* K12 and antibiotic-resistant bacteria

ABSTRACT

The freshwater ecosystem has been depleted gradually by human activities along the rivers including the waste disposal, which will contaminate water ecosystem, and uncontrolled widely antibiotic use in aquacultural purpose. Fecal contamination and the presence of antibiotic-resistant bacteria can cause serious problems to people living along the lake and rivers. Therefore, this study aims to investigate the survival of *Escherichia coli* K12 using dialysis membrane and to detect the antibiotic-resistant bacteria (ABR) in Tonle Sap River (TSR), Mekong River (MR) and Basac River (BR). The physiochemical and microbiological characteristics of waters in these 3 sites were analyzed. Moreover, control (*E. coli* K12/PBS) and sample (*E. coli* K12/River water) conditions were placed on the surface of TSR, MR and BR and the survival of *E. coli* K12 was observed for 12 days. In addition, six antibiotics including ampicillin, meropenem, ciprofloxacin, vancomycin, kanamycin and tetracycline were used to detect the antibiotic-resistant bacteria in these 3 sites. As the result, physiochemical characteristics slightly affect the survival of *E. coli* K12 as the turbidity was higher which could block sunlight to get through the waters. Moreover, it was found that *E. coli* K12 could survive only 8 days in TSR and they continued to survive until day 10 and died out in day 12 in MR and BR. The detection of ARB showed that high numbers of bacteria ranging from 3×10^2 to 2.31×10^4 CFU/mL were resistant to all antibiotics used. Among these 3 sites, ARB were detected in high amount in TSR.

1. INTRODUCTION

Despite the fact that Tonle Sap Lake has enormous significant for Cambodia in term of hydrological system, diverse aquatic system, aquatic food source to human, and also the place where fishes can breed, its water quality is reported to be declined because of the discharge of agricultural wastes, urban area wastes and human activities[1,2]. For instance, floating villages on Tonle Sap Lake (TSL) is reported to be a major fecal contamination to the lake and also to rivers because of poor household sanitation system.

Escherichia coli is known as fecal indicator organism and some strains of this specie are pathogenic bacteria causing serious public health concern. However, the fate of *E. coli* in natural environment has not been well explained when they are released to the water environment which can lead to the public health problems[3]. And *E. coli* K12 is well known as model organism that is extensively studied to understand particular biological phenomena.

The spread of antibiotic-resistant bacteria in the environment may compromise the ability to treat microbial infections and has become an increasing challenge to public health[4]. In order to complete microbial risk assessments and develop policies to protect public health, understanding the fate of fecal indicator in the lake and river waters, and antibiotic- resistant bacteria (ARB) are needed.

The objective of this study was therefore to investigate the survival of *E. coli* K12 and to detect the antibiotic-resistant bacteria in Tonle Sap, Mekong and Basac rivers in Cambodia.

2. METHOD

2.1. Physicochemical and microbiological analysis

The physicochemical and microbiological characteristics of waters in 3 sites (**Fig. 1**) were first analyzed during rainy season in July 2017. For microbiological analysis, *E. coli*, coliforms and other Gram-negative bacteria were investigated using Chromocult® coliform agar.

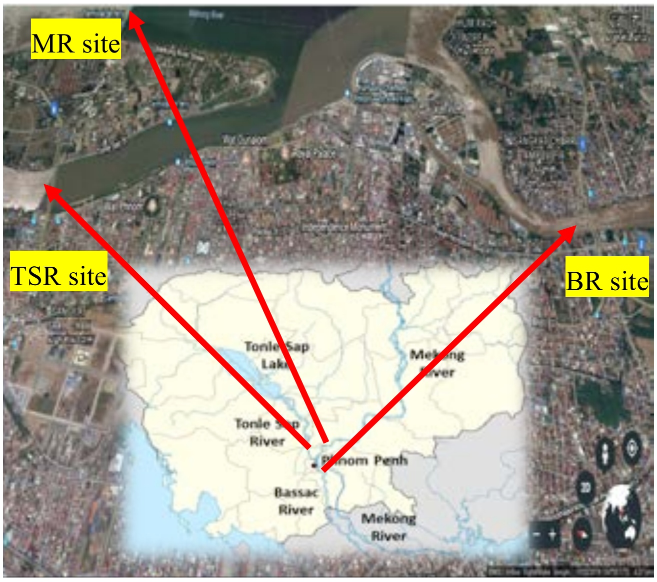


Fig. 1 Study sites

2.2. Experiments on survival of *E. coli* K12

Float-A-Lyzer® G2 was used as a dialysis membrane with molecular weight cut off (MWCO) from 0.1-1,000 kilo Dalton. The overnight culture of *E. coli* K12 strain W3110 was centrifuged at 4000 rpm for 15 minutes and washed twice with Phosphate Buffer Saline (PBS). Then the final cells were diluted 10 times with PBS in order to start field experiment with 10^8 or 10^7 CFU/mL There were 6 experimental conditions as shown in **Table 1**. The control was done by adding 1 mL of bacterial suspension inside the dialysis membrane with 9 mL of PBS while the outside of membrane, 9 mL of river water was added with 1mL of bacterial suspension. The dialysis membranes were placed on the surface of waters for 12 days and 1 ml samples were collected on day 0, 1, 2, 4, 6, 8, 10 and 12 for microbial counts by spread plate technique and incubated at 37°C for 48h. The numbers of colonies with salmon to red (coliforms), blue to violet (*E. coli*) and white colonies (other Gram-negative bacteria) were counted and calculated as CFU/mL.

Table 1 Experimental conditions of *E. coli* K12

Condition	Bacteria	Inside	Outside	Site
1	*E. coli* K12	PBS	RW	TSR
2	*E. coli* K12	RW		
3	*E. coli* K12	PBS	RW	MR
4	*E. coli* K12	RW		
5	*E. coli* K12	PBS	RW	BR
6	*E. coli* K12	RW		

2.3. Detection of antibiotic-resistant bacteria

River water samples were also collected from the 3 sites above. The colonies that could grow on R2A agar plates containing ampicillin (50 µg/mL), meropenem (5 µg/mL), kanamycin (50 µg/mL), ciprofloxacin (5 µg/mL), vancomycin (30 µg/mL) and tetracycline (50 µg/mL) were ARB. And cycloheximide was added to these plates in order to inhibit the growth of yeasts and molds.

3. RESULTS

3.1. Physicochemical and biological characteristics of river waters

The physicochemical and microbiological characteristics of TSR, MR and BR are shown in **Table 2**. The turbidity of these rivers was a bit different between them. In addition, *E. coli* were present highest in TSR, 1.27×10^3 CFU/mL, followed by BR, 7.67×10^2 CFU/mL, and MR, 6×10^1 CFU/mL, was the lowest.

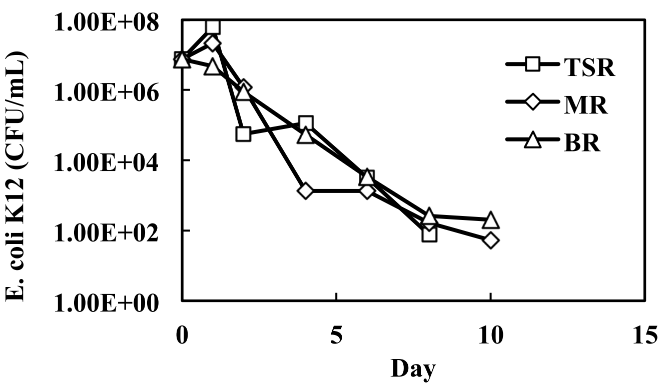
Table 2 Physiochemical and biological characteristics of waters in TSR, MR and BR

Parameter	TSR	MR	BR
pH	5.70 ± 0.02	5.87 ± 0.02	5.90 ± 0.01
EC (mS/m)	10.26 ± 0.01	10.52 ± 0.04	10.58 ± 0.02
Turbidity (NTU)	186.60 ± 1.25	250.00 ± 1.63	267.33 ± 1.25
TDS (ppm)	67.33 ± 0.47	69.33 ± 0.47	70.67 ± 0.47
TP (mg/L)	0.47 ± 0.00	0.43 ± 0.00	0.54 ± 0.01
TN (mg/L)	2.50 ± 0.01	2.40 ± 0.01	2.65 ± 0.00
E. coli (CFU/mL)	$1.27\times10^3 \pm 3.09$	$6\times10^1 \pm 2.45$	$7.67\times10^2 \pm 4.62$
Coliforms (CFU/mL)	$2.8\times10^3 \pm 8.83$	$1.60\times10^2 \pm 9.81$	$2.47\times10^3 \pm 1.70$
Other bacteria (CFU/mL)	$2.6\times10^3 \pm 11.43$	$3.07\times10^2 \pm 3.09$	$1.27\times10^3 \pm 3.09$

3.2. Comparison of the survival of *E. coli* K12 in TSR, MR and BR

The survival of *E. coli* K12 in 3 different sites with two different conditions (control and sample) is shown in **Fig. 2**. In day 1 of control condition, the concentration of *E. coli* K12 in TSR was higher than that in MR and BR, while *E. coli* K12 in BR were the lowest (**Fig. 2a**). However, the number of *E. coli* K12 in TSR became the lowest while those in MR were the highest in day 2. In contrast, their numbers were lowest in MR and highest in TSR in day 4. Moreover, there were similar concentrations of bacteria in these 3 sites in days 6 and 8. On the other hand, *E. coli* K12 in TSR site were not detected in day 10. In **Fig. 2b**, the numbers of *E. coli* K12 in day 0 were not different between these 3 sites. It was noticed that *E. coli* K12 in MR were highest in day 1 and they were highest in BR in day 2. In day 6, their numbers were similar among these 3 sites. Despite of this, their number was high in MR while they were similar in TSR and BR in day 8. Finally, only there was no *E. coli* K12 detected in TSR but they still be detected in MR and BR in day 10. And their number was high in MR than in BR.

(a)



(b)

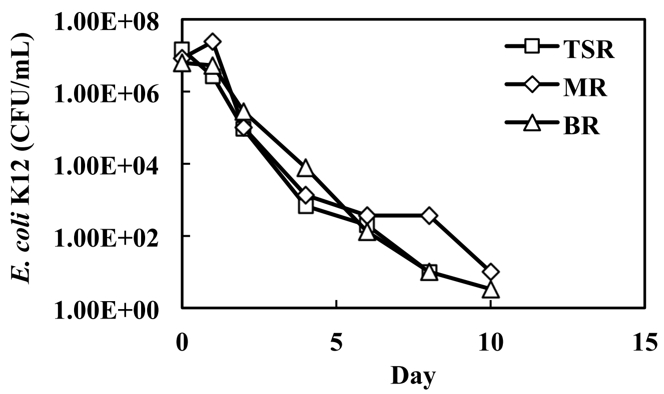


Fig. 2 Survival of *E. coli* K12 in TSR, MR and BR: (a) control and (b) sample

3.3. Comparison of antibiotic-resistant bacteria TSR, MR and BR

The numbers of bacteria resistant to all antibiotics were found to be high ranging from 3×10^2 to 2.31×10^4 CFU/mL. Among these 3 sites, the bacteria resistant to ampicillin, meropenem and tetracycline were reported to be the highest in TSR and lowest in MR. Moreover, the bacteria resistant to ciprofloxacin, kanamycin and vancomycin were found to be highest in TSR and lowest in BR. For the controls which contained only cycloheximide, the numbers of bacteria were 2.55×10^5 CFU/mL, 6.59×10^3 CFU/mL and 9×10^3 CFU/ml in TSR, MR and BR, respectively.

4. DISCUSSION

Comparing to drinking water quality standard by Institute of Standard of Cambodia (2004), which allowed only other bacteria to be present in the range of 100 CFU/mL while Coliforms and *E. coli* are not allowed to be present, 0 CFU/mL. It could be interpreted that it would affect to people who consumed these waters directly without boiling or any treatment, especially referred to those people who are living along the rivers.

In our previous study, the survival experiments were conducted only 6 days. The result showed that *E. coli* K12 in TSL lost their presence on day 6 while on that day, they were still detected for all conditions conducted in TSR. Therefore, TSR provided better conditions for the survival of *E. coli* than TSL. In this recent study, it also showed that *E. coli* K12 in TSR could survive until day 8 and *E. coli* K12 in MR and BR could survive until day 10. It is suggested that MR and BR provide better conditions than TSR and TSL. Therefore, the survival of *E. coli* K12 is not short and their presence can be harmful to people who use these waters without treatment before drinking.

Among the 3 sites, the bacteria in TSR were resistant to all antibiotics with the highest concentration. This result may be because of the multiple usages of antibiotics along the Tonle Sap River and Tonle Sap Great Lake for aquacultural purpose or left over after human consumption to treat diseases.

5. CONCLUSION

The physicochemical characteristics of waters did not affect the survival of *E. coli* K12 in these 3 sites even though the turbidity was reported to be highest in BR that could block the sunlight into the water. In TSR, *E. coli* K12 could survive for 8 days only but they continued to survive until day 10 in both MR and BR. In addition, the bacteria present in these 3 waters were resistant to all antibiotics used in this study with high concentrations from 3×10^2 to 2.31×10^4 CFU/mL. And the highest concentration of all antibiotic-resistant bacteria was found in TSR.

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Temporal Dynamics of Water Quality in Tonle Sap Lake in Kampong Loung, Cambodia, Based on Historical Data

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Keywords: temporal variation, Spearman, Mann-Kandall, Sen's slope, cluster analysis.

ABSTRACT

Assessment of seasonal changes in surface water quality is an important aspect for evaluating temporal variations of river pollution due to natural or resulting from inputs of point and non-point sources. In this study, the total of 16 surface water quality parameters was collected from 1995 to 2010 at the Kampong Loung, the Southern part of the largest freshwater source in South East Asia, Tonle Sap Lake by MoWRAM. Hierarchical agglomerative clustering technic was applied to assess classification of the data. The trend analysis was done using the Mann-Kandall and Sen's slop estimator based on cluster and monthly average from 1995 to 2010. The analysis reveals 3 classes were found with the level of (Dlink/Dmax) x100 < 25%. The significant trend was detected significance for most parameters except CODMN for the monthly average trend analysis. The positive trend was determined in the first cluster. The test found temperature has significantly increased which lead to increasing concentration Ca, K, and TOTP. The reason of increase TSS due to the movement of floating village and busy boat navigation. The study is crucial for understanding for future sampling as well as supporting a decision on lake management.

1. INTRODUCTION

Surface water quality is the vulnerable to pollution from runoff which contributed by nature or human activities [1]. The water quality of water resource is an unending issue. Conducting the water quality assessment for long-term water quality is the main challenging problem to research around the globe. In the last decade, water quality inspection has rapidly increased to raise awareness for a better understanding of its temporal corresponding the future study and evaluation of water quality characteristic. However, the result has to be interpreted or simplified be useful to the public [2]. The multivariate statistical method is widely used to better understanding of water quality characteristic and its source. The methods are known as parametric and non-parametric. The parametric method is more powerful than non-parametric but its required normal distributed time series data and independent. The method is used to determine the linearity of the regression using a t-test to compute the significance level of slope. The Mann-Kendall's test is the most common method applied in non-parametric parameters. The increasing and decreasing can be computed by the test [2-3].

The presente study aimed to classify the sampling frequency for the future monitoring program in the Kampong Loung using hierarchical agglomerative cluster analysis and to assess trend of water quality by monthly average and trend of water quality in each cluster using Mann-Kendall Sen's slop test.

2. METHOD

Study Site: The Tonle Sap Lake is located in central part of Cambodia in **Fig 1**. It is an integral part of the Mekong River system. It is the largest permanent freshwater body in Southeast Asia and an important natural reservoir for the Mekong River. This phenomenon occurs annually in May–June. In September–October, when the water level in the Mekong starts to recede, this stored water starts to drain back into the lower Mekong and Bassac Rivers Fig.1.

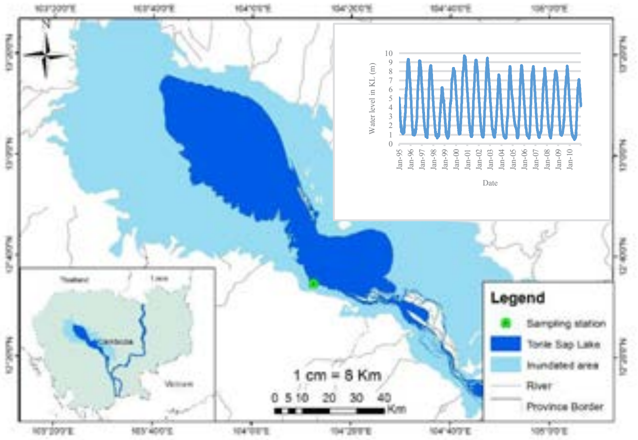

Fig. 1 Location of Kampong Luong station and its fluctuation of water level during 1995-2010

Hierarchical Agglomerative Cluster Analysis was used to group sampling frequency. The monthly average of water quality parameters from 1995 to 2010 were used.

In order to detect a trend, Mann-Kendall Seasonal Test was applied on 16 water quality parameters from 1995 to 2010. The level of the positive or negative trend was found by means of Sen's slope. The targeted parameters were: water temperature (TEMP), pH, Total suspended solids (TSS), conductivity (COND), calcium (Ca^{+2}), magnesium (Mg^{+2}), sodium, (Na^{+}), potassium (K^{+}), Alkalinity (ALK), chloride (Cl), sulfate (SO_4^{-2}), Nitrates (NO_3^{-2}), Ammoniacal-nitrogen (NH4-N), total phosphates (TOTP), dissolved oxygen (DO), chemical oxygen demand of permanganate (CODMn).

3. RESULT AND DISCUSSION

Temporal Cluster Analysis: In cluster 1, the clustering showing the convenient time by combining the 5 months together starting from February to June which corresponding to the lowest season (dry season). Cluster 2 (September to November) and cluster 3 (December, January, July and August) **Fig. 2**. With consistent to the peak and receding period (water in the lake start to rise to peak in September to November and start to recede in December, January, July and August). This suggest that for water quality assessment, only one time in each the cluster is adequate enough to represent water quality data in the study area (i.e. sampling frequencies can be done 3 times a year in Kampong Loung). Among all clusters, most water quality parameters in cluster 1 found consist of positive trend while cluster 2 has no trend and a negative trend found in cluster 3.

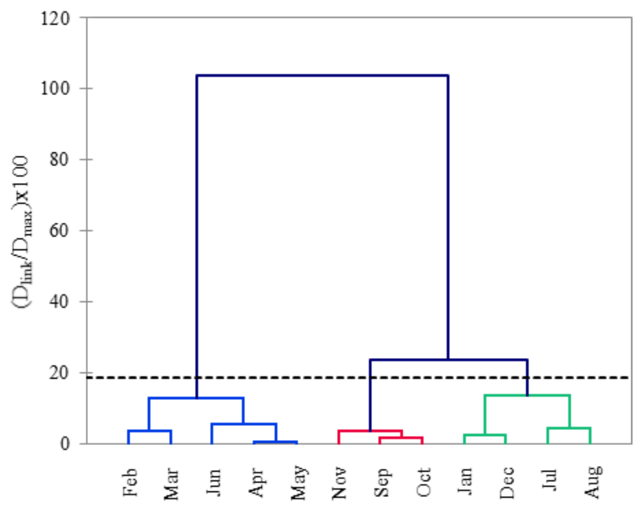

Fig. 2 Dendrogram showing cluster analysis of sampling frequency

Temporal variability of monthly water quality: To understand the temporal variability of monthly water quality, result from analysis was used to group the monthly water quality. The average monthly water quality was shown in **Table 1**. The average monthly water quality supported the clustering results by showing the similar of monthly average value of each parameters. From February to June (cluster group 1, water lever was from 1-2.4 meters. Sept to November (cluster group 2). The rising and falling months (cluster group 3: December, January, July and August), water level were 3.5 to 5.7 meters. Temporal variation of water level resulted in the temporal variation of water quality variables in this area as shown in **Table 1**.

Table 1 Monthly water quality in Kampong Luong by cluster analysis.

Parameters	Cluster 1					Cluster 2			Cluster 3			
Month	Feb	Mar	Apr	May	Jun	Sep	Oct	Nov	Dec	Jan	Jul	Aug
WL_m	2.43	1.55	1.02	1.02	1.57	8.07	8.43	7.49	5.64	4.05	3.59	5.77
TEMP_C	29.5	30.6	31.7	31.3	31.1	29.9	30.6	29.6	28.0	28.4	30.7	29.7
pH	7.30	6.96	6.89	6.99	6.97	6.91	6.98	6.86	6.83	6.91	7.10	7.09
TSS_mgL	37.1	100.9	249.0	247.7	173.5	21.6	7.3	6.1	15.9	27.4	24.8	39.6
COND_mSm	8.12	9.03	8.48	8.71	10.97	9.60	8.56	7.44	7.77	8.69	11.16	10.50
Ca_meqL	0.38	0.33	0.27	0.30	0.44	0.53	0.44	0.37	0.34	0.39	0.53	0.57
Mg_meqL	0.17	0.20	0.19	0.16	0.24	0.25	0.22	0.20	0.22	0.23	0.25	0.25
Na_meqL	0.24	0.26	0.35	0.35	0.34	0.17	0.17	0.16	0.19	0.24	0.26	0.23
K_meqL	0.05	0.05	0.06	0.07	0.05	0.03	0.04	0.03	0.04	0.04	0.04	0.04
ALK_meqL	0.61	0.56	0.50	0.46	0.63	0.80	0.68	0.57	0.58	0.66	0.73	0.84
Cl_meqL	0.09	0.10	0.12	0.16	0.14	0.06	0.06	0.05	0.08	0.09	0.10	0.08
SO4_meqL	0.11	0.12	0.17	0.22	0.27	0.11	0.12	0.11	0.13	0.10	0.21	0.14
NO3_mgL	0.06	0.39	0.60	0.55	0.26	0.08	0.05	0.07	0.07	0.15	0.18	0.16
NH4-N_mgL	0.08	0.14	0.11	0.05	0.06	0.09	0.07	0.06	0.08	0.08	0.10	0.09
TOTP_mgL	0.05	0.11	0.17	0.26	0.15	0.03	0.04	0.03	0.09	0.09	0.06	0.08
DO_mgL	6.70	6.95	5.29	5.96	5.63	5.73	5.70	6.59	5.98	6.27	6.19	5.30
CODMn_mgL	5.65	5.69	5.83	5.06	4.23	3.17	3.09	3.59	4.37	4.78	3.57	2.59

Trend analysis: The test was applied on non-parametric parameters to study the significance of trend at on each month. The result shows the trend detections on monthly average **Table 2** and by the cluster.

The study reveals that positive trend was detected significance for COND, K, SO_4 with the mean value of Sen's slope shown in **Table 2**. Among 16 parameters, SO4 has the most frequent significance trend compare to the other variable (clearly detected as 66% over the year) and COND has significance trend 58% over the year. The increasing of SO4 mainly contributed by SO4 base fertilizer of agricultural land nearby the station (i.e. agricultural land increases its area and consumes more fertilizer). The negative trend was found significance with the average of Sen's slope -0.006 and -0.015 for May and

July respectively for NH4-N. The trend also spotted with CODMN (41% over the year). There is no trend detected for Temp and TOTP. For the rest of parameters such as pH, TSS, Ca, Mg, Na, K, Alk, Cl, NO3, and DO, the trend was detected in less than 25% over the year.

The trend of total suspended solid shows the significance at all level (0.01, 0.05 and 0.11) in February, September and August. TSS has a strong correlation with turbidity.

Therefore, the increase of concentration can be clearly identified. This might cause by the floating village which moved to the concerned area during the lowest season (February) lead to high turbidity. Among all clusters, most water quality parameters in cluster 1 found consist of the positive trend while cluster 2 has no trend and a negative trend of CODMn found in cluster 3. The output of trend study by cluster can be found in **Table 1**. In cluster 1, the temperature was detected increase which leads to significantly increase in Ca, K and TOTP concentration in the lake. Because of lower water level in February, March, April, May and June, the turbidity has significantly increased.

4. CONCLUSION

This study present temporal analyzing of 16 water quality variables from 1995 to 2010 in Kampong Loung, Southern part of Tonle Sap Great Lake. The result from cluster shows the sampling period can be classified into 3 clusters corresponding to three sampling frequency. Water quality sampling can be done in one of the following month, February, March, June, April, or May for cluster 1. In cluster 2, the sampling frequency is done either November, September or October. The third cluster was found and can be grouped January, December, July and August together. The Mann-Kendall and Sen's Slope estimator test reveal both the negative and positive trend. In monthly average, SO4 was found positively increase 66% over the year which contributed by the fertilizer consumed in the paddy field. Moreover, TSS also found a significant increase in February due to the movement of floating house and busier boat traffic. The test shows most water quality variables in cluster 1 such as Temp, TSS, Na, P, Cl, and TP has a significant positive trend. The concentration of the parameters is increase due to the increase in water temperature. The study provides better and understanding of future sampling. Moreover, it has a positive impact to support a decision on lake management.

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Table 2 Trend direction and magnitude of monthly water quality in Kampong Luong from 1995-2010

Month	Temp	pH	TSS	COND	Ca	Mg	Na	K	ALK	Cl	SO4	NO3	NH4-N	TOTP	DO	CODMN
Jan	n.t.	n.t.	n.t.	0.235	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.006	n.t.	n.t.	n.t.	n.t.	n.t.
Feb	n.t.	n.t.	2.36	0.190	n.t.	n.t.	n.t.	n.t.	n.t.	0.005	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Mar	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.013	n.t.	n.t.	n.t.	n.t.	-0.258
Apr	n.t.	n.t.	n.t.	0.3377	0.012	0.008	n.t.	n.t.	0.012	0.011	0.014	n.t.	n.t.	n.t.	-0.197	n.t.
May	n.t.	n.t.	n.t.	0.431	0.028	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	-0.006	n.t.	n.t.	-0.224
Jun	n.t.	n.t.	n.t.		n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Jul	n.t.	n.t.	n.t.	0.219	0.020	n.t.	0.009	n.t.	0.022	n.t.	n.t.	n.t.	-0.015	n.t.	n.t.	-0.309
Aug	n.t.	n.t.	3.874	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.007	n.t.	n.t.	n.t.	n.t.	n.t.
Sep	n.t.	-0.043	2.507	n.t.	n.t.	n.t.	n.t.	n.t.	-0.008	n.t.	0.007	n.t.	n.t.	n.t.	n.t.	n.t.
Oct	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.01	-0.003	n.t.	n.t.	n.t.	-0.125
Nov	n.t.	n.t.	n.t.	0.129	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.007	n.t.	n.t.	n.t.	n.t.	-0.170
Dec	n.t.	n.t.	n.t.	0.181	n.t.	n.t.	0.004	0.0010	n.t.	0.003	0.009	n.t.	n.t.	n.t.	n.t.	n.t.

+	significance at alpha=0.1
*	significance at alpha=0.05
**	significance at alpha=0.01
n.t.	no trend

O3-37

猪苗代湖における湖水水質の長期変動

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キーワード：水質汚濁, 栄養塩動態, 一次生産

抄録

猪苗代湖では，1990年代中頃から始まった湖水の急激なpH中性化とともに，CODや大腸菌群数の上昇に代表される水質の悪化が顕在化している。本研究では，この原因を調べるために，福島県が保有している水質データを集計して湖水水質の長期変動傾向を解析した。その結果，猪苗代湖湖水におけるCODおよび大腸菌群数の上昇は，中性化後に湖内の生物生産量が飛躍的に増大したことに起因する可能性が高いことがわかった。また，この生物生産量の増大から，猪苗代湖における栄養塩類動態が pH 上昇とともに変化した可能性が考えられた。今後の猪苗代湖の水質改善策を検討するにあたって，湖水水質および流入河川水質の長期モニタリングの継続に加えて，猪苗代湖内の有機物および栄養塩類動態，流入河川から汚濁負荷実態に関する詳細な調査・研究の実施が必須であると考えられた。

1.　はじめに

猪苗代湖は，酸栄養湖に分類されており，1995 年頃までは，湖水の pH が 5 前後の弱酸性を示していた。しかし，2010 年には 6.8 まで上昇し，以降は pH がほぼ中性を維持し続けている。また，この pH 上昇に伴い，猪苗代湖では 2002 年頃から COD が上昇し，さらに，2006 年以降は，表層水の大腸菌群数が環境基準である（1000MPN 100mL^{-1}）を超過し続けるなど，水質の悪化が顕在化している。

大腸菌群数の上昇は，猪苗代湖湖水内において，従属栄養細菌類が中性化に伴って増加していること示唆している。同時に，その増加を支えるだけの炭素基質が湖水内に存在することも示唆している。したがって，その原因を明らかにするためには，有機物生産に関わる水質項目の長期変動を調べる必要がある。

福島県では，1970 年代後半から猪苗代湖およびその流入河川において水質データを取得し，福島県水質年報や福島県環境センター年報という形で公表してきた。しかし，これらのデータを使用して水質の長期変動傾向を解析した例は，流入河川からの酸性物質負荷量についての報告例[1]が 1 件存在するのみであり，湖水中の有機物生産の面から長期変動を解析して水質悪化の原因について考察した例については今のところ見当たらない。

そこで本研究では，福島県がこれまでに蓄積してきた猪苗代湖湖水および流入河川水の水質データについてとりまとめをおこない，猪苗代湖湖水のCODおよび大腸菌群数上昇の原因について考察した。

2.　方法

2.1.　猪苗代湖の概要

猪苗代湖は，福島県のほぼ中央に位置する，集水域面積 820.2 km^2, 湖面積 103.3 km^2（日本第 4 位），最大深度 94.6 m, 平均深度 51.5 m の湖である。おもな流入河川は，北岸へ流入する酸性河川の長瀬川であり，河川からの総流入量の 50%以上を占めている。猪苗代湖への河川からの流入水量は約 31.7 m^3 s^{-1}, 平均滞留時間は 5.4 年である[2]。

長瀬川河川水が酸性を示す原因は，長瀬川の支流である酸川の上流部に硫黄川が存在するためである。硫黄川は，安達太良山の沼ノ平火口が起点であり，流下に伴い酸性の温泉水（硫黄泉）や旧硫黄鉱山の坑道からの流出水が合流しており，河川水の pH が 2 前後の強酸性を示すのが特徴である。

2.2.　調査地点

本研究では，猪苗代湖湖水水質の長期変動を解析するための調査地点として，環境基準点である猪苗代湖湖心の水質データを使用した。調査地点の位置は，図 1 に示した。

2.3.　調査期間

前節に記述した調査地点の水質データを福島県水質年報（1974～2014）[3]および福島県環境センター年報（2002～2016）[4]から抜粋して集計した。

3.　結果

3.1. 湖心表層水における pH，アルカリ度およびイオンバランスの長期変動

猪苗代湖湖心における表層水の pH は，1995 年頃から急激な上昇が始まっている。しかし，1974 年から 1995 年の間にも緩やかな上昇が見られた（図 2 (a)）。

表層水のアルカリ度は，pH が急激に上昇している 2002～2016 年の 14 年間の間に，約 3 倍（1.4 mg L^{-1} から 5 mg L^{-1}）の増加が見られた（図 2 (b)）。

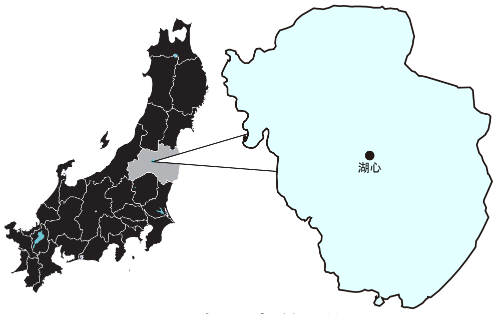

図 1　調査地点位置図

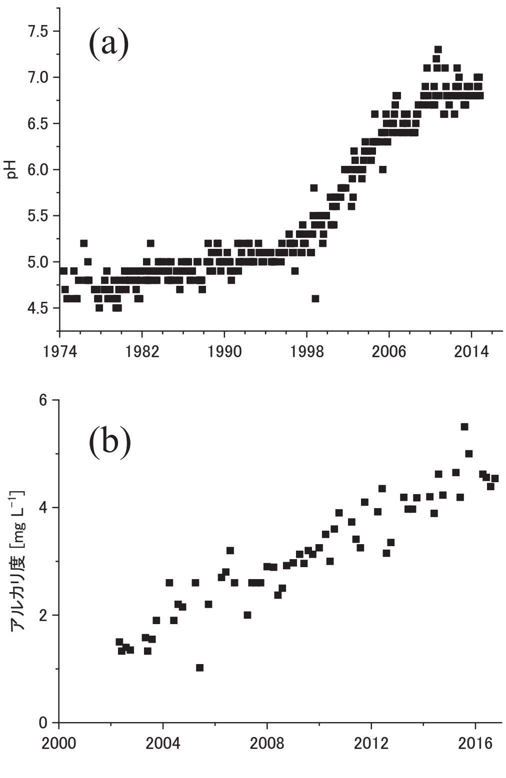

図 2　湖心表層水における(a) pH および(b) アルカリ度の時系列変化

3.2. 動植物プランクトンの細胞密度の長期変動

湖水 pH の中性化以前と以後の動植物プランクトンの細胞密度には大幅な増加が見られた（図 3）。植物プランクトンの細胞密度は，湖水 pH が 5 以下だった 1980～1985 年頃には 10^3 cells m^{-3} 以下だったのに対し，pH が 6.8 になった 2010 年には 10^6～10^8 cells m^{-3} まで増加していた。動物プランクトンの細胞密度も植物プランクトンと同様に 10^2 cells m^{-3} から 10^4 cells m^{-3} に増加していた。

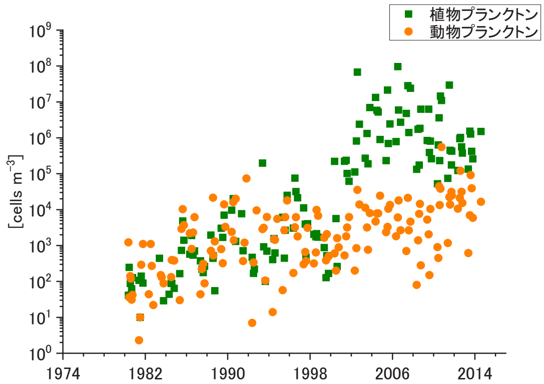

図 3　湖心表層水における動植物プランクトンの時系列変化

3.3. 全窒素濃度の長期変動

猪苗代湖湖水の全窒素濃度は，1982～2014 年の間では 0.17～0.5 mg L^{-1} の範囲で推移していた（図 4）。

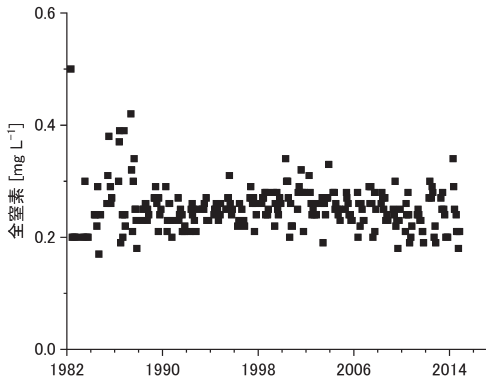

図 4　湖心表層水における全窒素濃度の時系列変化

4. 考察

猪苗代湖の表層水において，動植物プランクトンの細胞密度が湖水 pH の中性化以後に大幅に上昇した。これは，湖水における一次生産量が中性化以後に急激に増加したことを示しており，これが河川からの流入負荷に上乗せされる形になり，COD が上昇したと考えられた。さらに，植物プランクトンの細胞や分泌物には多量の糖質が含まれること[5,6]や細菌類によって優先的に分解されること[5,6]が明らかになっている。猪苗代湖湖水には，これらを炭素基質としている従属栄養細菌類が中性化以後に増加している可能性が考えられた。大腸菌群数の上昇は，従属栄養細菌類のバイオマスの増加を

示唆するものと推察された。

表層水において，湖水 pH の中性化以後に植物プランクトンが急激に増殖した原因は，湖水の pH 上昇によって生体毒性が低下したことに加えて，光合成の炭素基質である重炭酸イオンの増加にもあると考えられた。また，猪苗代湖では，全窒素に関して環境基準の類型指定はされていないが，仮に全リンと同じ類型をあてはめた場合，環境基準値の 0.2 mg L^{-1} を達成できておらず，高濃度の状態が継続している。これは，猪苗代湖への栄養塩類の負荷が大きい可能性を示唆するものと考えられる。猪苗代湖では，酸性の河川から供給される鉄やアルミニウム水酸化物を主成分とするフロックによってリンが吸着されることなどによって湖水中のリン濃度が極端に低い状態にあり，それが生物生産を抑制してきたと考えられている[7]。しかし，湖水の pH が弱酸性から中性に変化する過程において，大きな時間差がなく細胞密度が増加したことから，湖水pHの上昇とともに湖水中の栄養塩類動態が大きく変化した可能性が考えられた。

5. 結論

長期モニタリング結果の解析から，猪苗代湖湖水におけるCODおよび大腸菌群数の上昇は，湖水pHが弱酸性だった時代には生物生産が著しく抑制された状態にあったものが，中性化後に湖内の生物生産量が飛躍的に増大したことに起因する可能性が高いことがわかった。また，この生物生産量の増大から，湖水中での栄養塩類動態が pH 上昇とともに変化した可能性が考えられた。今後の猪苗代湖の水質改善策を検討するにあたって，湖水水質および流入河川水質の長期モニタリングの継続に加えて，猪苗代湖内の有機物および栄養塩類動態，流入河川からの汚濁負荷実態に関する詳細な調査・研究の実施が必要と考えられた。

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O3-38

Water Quality of Five Selected Lakes in Southern Luzon, Philippines

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Keywords: Physical Parameters, Chemical Parameters, Water Quality, Lakes

ABSTRACT

The study focuses on important lakes in the Philippines such as Bunot Lake, Taal Lake, Tikub Lake, Bato Lake and Buhi Lake. This paper aimed to describe the physical and chemical parameter analyses of five selected lakes in Southern Luzon, Philippines. Three replicates of water samples were collected (phosphate and TSS); and others were measured on sites from five lakes (January to March 2017). The physical parameter included water temperature, pH and turbidity, whereas the chemical parameters included conductivity, dissolved oxygen (DO), phosphate, ammonia, and total suspended solids (TSS). These five selected lakes revealed that conductivity, turbidity, TSS, phosphate and ammonia content exceeds the DENR Administrative Order (DAO 2016-08) range. The results are significantly different between 0.22±0.00 to 1779.42±59.41 mg/L for conductivity; 8.42±1.00 to 50.23±23.02 NTU for turbidity; 1±0.00 to 96±76.68 mg/L for TSS; 0.07±0.01 to 1.22±0.03 mg/L for phosphate; and lastly, 0.27±0.04 to 1.47±0.35 mg/L for ammonia. Taal Lake has the largest variation of conductivity, phosphate and ammonia. Bato Lake has the largest variation of turbidity and TSS. Bunot Lake has the largest variation of phosphate and ammonia because of anthropogenic activities. The extremely high content of TSS and turbidity has caused poor and stressful conditions for the aquatic life of lakes. Proper management, planning and monitoring is required to prevent further damage of the lake ecosystem from different anthropogenic activities.

1. INTRODUCTION

In the Philippines, where there is an increasing number of polluted aquatic ecosystems [1]. Researchers and studies directed to restoring, rehabilitating and managing deteriorating water bodies are gradually getting priority.

Lakes are very valuable resources that provide people with prime opportunities for recreation, tourism, and residential living. Lakes are also home for a huge diversity of flora and fauna in the Philippines.

Through the years, DENR-EMB has proposed water quality parameters – Dissolved Oxygen (DO), pH, Total Suspended Solids (TSS), turbidity, conductivity, water temperature, phosphate and ammonia which are suitable for temperate regions [2]. Water quality parameters are based on DAO 2016-08.

The findings of this research illustrate the potential significance of the lakes in Bicol Region since the production of this fishes known as *Mistichthys luzonensis* or also known as "sinarapan", the world's smallest fish that can be only seen in the Philippines particularly in Lake Bato and Lake Buhi. In Taal Lake which is situated in Talisay, Batangas – this is known for the home of the lowest active volcano and the only freshwater sardinella (*Sardinella tawilis*) in the world. In Tikub Lake which is located in Tiaong, Quezon, it is still unexplored and it has the potential to be one of the tourist destinations in the Philippines. Lastly, the Bunot Lake in San Pablo, Laguna this lake is known for its cultured tilapia and fish pens for *Nilotica* fingerlings.

Therefore, nowadays the solution of water quality and recovery of the multiple functions of water system have become the key issues for environmental biologists. This study aimed to describe the physical and chemical parameter analyses of five selected lakes in Southern Luzon, Philippines.

2. METHOD

Fig 1. Location of the sampling sites in Southern Luzon, Philippines.
Source: Google map (2018)

Location and Description of the Study Area

Luzon is the largest and most populous island in the Philippines. It is also ranked 15th largest in the world in terms of land area. The study was conducted in two regions of Southern Luzon. First is in Region 4A (CALABARZON: Cavite, *Laguna*, *Batangas*, Rizal and *Quezon*), and second is in Region 5 (Bicol Region: Albay, Camarines Norte, *Camarines Sur*, Catanduanes, Masbate and Sorsogon). Every sampling site is divided into four quadrants.

Sampling Methods

Twelve sampling sites (4 quadrants), 1km from each other were selected in each sampling location. Water temperature, DO, conductivity, turbidity, pH, turbidity and ammonia were measured onsite using a pre-calibrated Horiba (model: U-10) water quality checker. The probes were immersed in water and the parameter readings were noted after measurement stabilization.

Water samples were also collected for each sampling sites for the parameters that were not measured onsite. Sterile bottles were used to contain water samples for the TSS and Phosphate. These two are brought to Department of Environment and Natural Resources – Environmental Management Bureau (DENR-EMB) Region 4-A Laboratory for the analyses test. Method of analysis for Phosphate (as Ortho) is Ascorbic Acid Method and for TSS is Gravimetric Method [3]

3. RESULTS

organic particles. The turbidity values of the five lakes varied from 8.42±1.00 NTU to50.23±23.02 NTU, which exceeds the maximum standard of Minnesota Pollution Control Agency (25 NTU) [4]. A significant difference was found between the Bunot Lake (8.42±1.00 NTU) and Bato Lake (50.23±23.02 NTU). pH levels of this study is within the standard limit of DAO 2016-08.

The water conductivity of the surface water varied from 0.22±0.00 µS/cm to 1779.42±59.41 µS/cm. The only lake which exceeds the maximum limit of US EPA in conductivity was Taal Lake which has the value of 1779.42 µS/cm. The TSS included silts and clays, which cause the brownish color of the lake. The lakes in Southern Luzon ranged from 1±0.00 mg/L to 96±76.68 mg/L, which exceeds the DAO 2016-08 limit for Class C. The smallest value of TSS at Taal Lake (1±0.00 mg/L) and the greatest value at Bato Lake (96±76.68 mg/L). The TSS for the Southern Luzon was significantly different between Taal and Bato Lake.

Phosphate values range from 0.11±0.30 mg/L to 0.77±0.01 mg/L, which is alarming that exceeds in the DAO 2016-08 limit for Class B and C for Taal and Bunot Lake. The DO levels of the lakes varied from 5.48±0.52 mg/L to 7.97±0.21 mg/L which is within the DAO 2016-08 limit. The maximum value of DO was detected at Buhi Lake (7.97±0.21 mg/L) and the lowest DO value was detected at Tikub Lake (5.48±0.52 mg/L). The results for ammonia, exceeds for both Class B and C limit: for Bunot Lake (1.47±0.35 mg/L), Taal Lake (1.03±0.39 mg/L), and Bato Lake (0.66±0.16 mg/L) but according to BFAR (Bureau of Fisheries and Aquatic Resources) the values are still safe for fishes.

Table 1. Mean value±SD for the Physical and Chemical Parameters of the Five Selected Lakes along the Southern Luzon, Philippines.

Parameters	Units	Lakes				
		Bunot (Laguna)	Taal (Batangas)	Tikub (Quezon)	Bato (Camsur)	Buhi (Camsur)
Temperature	°C	25.7±0.13	26.8±0.39	27.2±0.14	28.65±0.59	26.25±0.33
Turbidity	NTU	8.42±1.00	14.65±0.92	9.50±0.52	50.23±23.02*	16.26±17.59
pH	-	7.65±0.24	8.41±0.86	7.60±0.10	8.16±0.18	8.45±0.15
Conductivity	µS/cm	0.22±0.00	1779.42±59.41*	0.24±0.00	340.25±65.83	236.83±101.87
TSS	mg/L	7±2.75	1±0.00	1±0.29	96±76.68*	3±0.72
Phosphate	mg/L	1.22±0.03*	0.77±0.01*	0.07±0.01	0.24±0.13	0.11±0.30
DO	mg/L	7.43±0.26	6.78±0.46	5.48±0.52	7.51±0.64	7.97±0.21
Ammonia	mg/L	1.47±0.35*	1.03±0.39*	0.45±0.21	0.66±0.16*	0.27±0.04

Notes: * indicates that the lake's values are significantly different from those of every other lake (α<0.05)

Table 1 summarizes the mean±SD of the physical and chemical parameters from the five lakes along Southern Luzon, Philippines. The water temperature of the five lakes ranged from 25.7±0.13°C to 28.65±0.59°C. All the data of water temperature are within the standard limit of DENR. The turbidity is become high also in Bato Lake (50.23±23.02 NTU) because of the dipersion of suspended particles. It is caused by fine sediment and

4. DISCUSSION

These five lakes were classified into polluted and non-polluted lakes according to their classification, but after the results it turned out that rapid changes happened in these lakes. Before Taal Lake was classified at Class B, but due to certain results that above the maximum levels of parameters this lake fall into Class C.While Bunot and Bato Lake exceeds in the results for Class C. Tikub Lake maintains its Class, one of the non-polluted lake in the Philippines that has a huge potential of tourist destination.

The water temperature is the most important ecological factor because it controls the physiological behavior and distribution of organisms [5]. The highest turbidity value was observed in Bato Lake (50.23±23.02 NTU) and the lowest turbidity value was observed in Bunot Lake (8.42±1.00 NTU). High tubidity can significantly reduce the good quality of lakes and can harm aquatic organisms by reducing food supplies, affecting gill functions and degrading spawning beds, it has been found out that the lake are turbid this means that there are a lot of suspended solid particles in the lake (soil from erosion, particulate matter, unconsumed fish feeds, and some plankton or microorganisms). The pH is one of the most important factors and serves as index for pollution. pH range between 6.5 to 9.0, where the fish grows well and fast in freshwater ecosystem [6].

The values water conductivity for the lakes is the following: low conductivity has 0 to 200 µS/cm; it means an indicator of pristine or background conditions. Mid-range conductivity is about 200 to 1000 µS/cm which is normal background for most freshwater. Conductivity outside this range could indicate that the water is not suitable for certain species of fish. High conductivity around 1000 to 10,000 µS/cm is an indicator of saline conditions [7]. TSS content between 1 and 96 mg/L on the mean values previously indicated the poor condition of aquatic ecosystems [8]. The high value of TSS during the season occurred because of the floating fine silt and detritus that was carried by the rainwater from the catchment [9]. Rapid changes and extremely high TSS values are stressful to fish and other aquatic organisms.

The low DO value may be because of high organic matter content. High organic matter limits primary production, and the senescence of phytoplankton increases microbial respiration that leads to the depletion of DO [8,9,10]. Meanwhile, ammonia (NH_3) is the major end product of protein catabolism excreted by fish and this is due to decomposed organic matters like unconsumed feeds and fertilizers, industrial and domestic wastes and decomposition of phytoplankton.

5. CONCLUSION

Physical and chemical analysis of the lakes in the study area demonstrated that the lakes was clean, with the exception that certain physicochemical parameters (such as TSS, turbidity, phosphate, ammonia and conductivity) had increased to extremely high levels that exceed the standards of DAO 2016-08, US EPA (conductivity) and Minnesota Pollution Control Agency (turbidity). As a result of Bunot Lake has the greatest variation in terms of Phosphate and Ammonia; Taal Lake has the greatest variation of Conductivity, Phosphate and Ammonia; and lastly, Bato Lake which has the highest value of Turbidity and TSS, and also exceeds in Ammonia.

Therefore, the researchers conclude that Taal Lake doesn't meet the criteria for Class B in terms of the parameters of Phosphate, Ammonia and Conductivity. Bato Lake is considered polluted for having high results of Turbidity and TSS of the water lake and ammonia results also is above the maximum value. Taal Lake and Bato Lake are both major lakes of the Philippines and also classified as big lakes, these two played an important role in the community, proper management and planning is required to prevent further damages of the lakes. It is noted that the recovery of the lakes from effects may take several years depending on the people's efforts and concerns as well as earth dynamics.

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O3-39

Genomic analysis of two odoriferous *Streptomyces* sp. isolated from tropical freshwater of Southeast Asia provide an insight into the identification of potential temperature sensor

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Keywords: Streptomyces, Temperature Sensor, Genome Sequence, Biosynthesis, Musty Odor

ABSTRACT

Musty odor compounds released have mostly been studied during the warmer season and well documented. Previous study successfully isolated two odoriferous *Streptomyces* sp. (S1 and S5) from tropical freshwater of Southeast Asia. Both isolates showed distinct characteristics against temperature difference (20°C and 30°C). S1 showed clear induction of geosmin at 30°C and none at 20°C contradict with S5 which produced higher amount of geosmin at 20°C compared to 30°C. In case of temperate isolate, they have thermosensing device to adapt temperature changes. However, S5 does not work to sense the temperature as it shows less sensitivity than S1 which lead to our hypothesis that S5 does not have the sensor for temperature stress. These isolate were then sequenced using Illumina HiSeq4000 and draft genome sequence yielded 7.79 Mb and 7.20 Mb genome size of S1 and S5, respectively. Since the genome size of S5 is smaller compared to S1, there might be some regulatory proteins which not available in the genome of S5 is/are available in S1 may be the possible temperature sensor. Here, we provide draft genome sequence analysis of both isolates which is very important for discovery more about this temperature sensor and to understand how the mechanisms involve in these isolate.

(204 words)

1. INTRODUCTION

Streptomyces is one of the most promising producer of secondary metabolites including antibiotics, immunosuppressant, extracellular enzymes and terpenoid metabolites [2, 4]. Geosmin, a terpenoid compound is widely known for its responsibility towards musty odor issue in freshwater systems worldwide. This odor issue is mostly prominent during cyanobacterial blooms in a warmer month in temperate regions. However, geosmin production by *Streptomyces* in tropical Asia has not yet been elucidated in details. Since tropical Asia is warm and stable throughout the year, the odor events can be expected to occur at any time of the year.

Our previous study investigated the effect of temperature on musty odor production by *Streptomyces* sp.[1]. Two isolates (S1 and S5) showed unique features to sense temperature stress. S1 shows high geosmin production at 30°C but no detection at 20°C which indicate that it has a good sense of temperature. But S5 does not work to sense the temperature as it produced higher amount of geosmin at 20°C compared to 30°C. S5 may possess some unique traits that allows it to produce large amount of geosmin at low temperature which contradicts with previous research of larger production at high temperature or in warmer seasons. We postulated that S5 did not contain the temperature sensor. Since S5 showed less sensitivity to temperature compared with S1, maybe some of the genes related to temperature sensor in S5 is missing. We need to see the difference between these two isolate and find which genes or proteins are available only in S1 and S5. If we find several genes in S1 but absent in S5, that genes supposed to be the temperature sensor. Therefore, draft genome analysis of these isolates were done in order to identify the missing or adding genomic content for temperature stress responded protein.

2. METHOD

General procedures.

Isolation of bacterial strains, temperature condition experiment and molecular experimental were discussed in details in our previous study [1].

DNA extraction, library preparation, sequencing, data preprocessing and assembly.

Bacterial DNA samples were extracted by using EZ-10 Spin Column Bacterial Genomic DNA Miniprep Kit according to the manufacturer's protocol. All the standard bioinformatics data analyses were provided by sequencing company (Genome Solution Sdn Bhd). Genome sequencing was performed using high-throughput Illumina

HiSeq4000 with a 150bp Paired End (PE) strategy. Sequencing reads were preprocessed, in order to use for de novo genome assembly and scaffolding. To predict putative genes in assembled genomes, an automated web-based tool RAST was used.

Bioinformatics analysis.

Ten homologous genes related to known germacradienol/geosmin synthase were obtained from GenBank (Q9X839, ABY50951, WP_003971162, WP_012382258, WP_014158016, WP_010983603, WP_018956438, WP_018515109, WP_014143690, and WP_012999852). BLAST algorithm was used for searching the homologous genes. Amino acid sequences alignment by MAFFT was done for searching the geosmin synthase conserved motifs in the isolates. To find unique protein sequences only in S1 (possible temperature sensor), USEARCH [3] analysis was used. S1 was used as query sequence and S5 was used as database. Then the sequences were analyzed using Pfam database to categorize the protein into several transcriptional regulators.

3. RESULTS

Genome properties

The draft genome of S1 contains 7.79 Mb with GC content of 72.33%, which containing 5792 protein-coding genes. As for S5, 7.20 Mb genomic size with 71.96% GC content and total of 6860 protein-coding genes. The summary of assembly statistics are shown in Table 1.

Table 1 Summary of the RAST-predicted genes and RNAs in the assembled genomes of S1 and S5

	S1	S5
Genome size (bp)	7,791,937	7,203,872
Number of Protein-coding Genes	5,792	6,860
Number of RNAs	72	70
GC content (%)	72.33	71.96
N50	33506	8483
Contig number	515	1463

Homology-based search

S1 and S5 shows high sequence similarity to the known germacradienol/geosmin synthase database with more than 60% similarity (data not shown). Bacterial terpene synthase typically displays two highly conserved Mg^{2+} binding motif: aspartate rich motif (**DD**XXX) and **NSE** triad. From the alignment results by MAFFT, N- and C-terminal of S1 and S5 (only S1 result was shown) shows significant sequence similarity to germacradienol/geosmin synthase to the homologous genes with the repetition of NSE motif **N**DLLT**S**RLHQF**E** at the N-terminal (Figure 1).

Predicted temperature sensor

Here, we want to identify which amino acid sequences is available only in S1 but absent in S5 which can be a candidate for the temperature sensor. From the USEARCH analysis, we found 5 query sequences that matched and 5787 sequences that not matched with the database (data not shown). From these 5787 unique protein sequences, we categorized each sequence into their possible transcriptional regulators (TRs) and separated it into two groups based on their regulated functions (Table 2). Group 1 comprised of TRs needed for the basic of cell functions and Group 2 TRs involved in either organism's metal homeostasis or in response to stressful situations [5].

Table 2 Transcriptional regulators (TRs) found in the unique sequences

Group 1	AraC, AsnC, DeoR, GntR, IclR, LacI, LuxR, ROK
Group 2	ArsR, MarR, MerR, TetR, LysR, PadR

4. DISCUSSION

Amino acid alignment shows that germacradienol/geosmin synthase has four Mg^{2+} binding motif, **DD**HFL and **N**DLF**S**YQR**E** in N-terminal and **DD**YYP and **N**DLF**S**YQK**E** in C-terminal which is essential for catalysis of geosmin [6]. Based on the amino acid alignment and conserved domains, we propound that S1 and S5 can serve as a germacradienol/geosmin synthase similar to already characterized *S. coelicolor* A3(2) SCO6073 and *S. avermitilis* SAV2163.

Comparison of the draft genome results between both isolates shows that S5 have smaller genome size compared to S1. Therefore, lead to our hypothesis that S5 does not have a good sensor for temperature. We therefore were intrigued that there might be some genes that supposed to be involved are absent in S5. USEARCH analysis with the 'search_exact' command have been performed on the amino acid sequences of S1 against of amino acid sequences of S5. Based on USEARCH results, 5787 amino acid sequences that have no matched within S5 database are defined as unique proteins. We managed to get these temperature sensor candidates. This result is very important for the screening of temperature sensor before proceed with the molecular experimental works. Bacteria that exposed to environmental stress need to adapt and response in order to survive by modifying their gene expression. One of the major players in this mechanism is a protein called transcription factors (TFs) which responsible for induction and repression of certain genes.

```
                 83          94  227           240  252                                283
Q9X839        ......FFFDDHFLEKYK........HLRNDLFSYQREVE........LETFFGCTTQEAADLVNDVLTSRLHQFEHTAF........
WP_012999852  ......FFFDDHFLELYK........HLRNDLFSYQREVE........LETFFGCGTQQAAETVNDILTSRLHQFEDTAL........
WP_010983603  ......FFFDDHFLETFK........HLRNDLFSYQREVE........LETFFGCTTQEAAETVNDILTSRLHQFEHTAL........
WP_003971162  ......FFFDDHFLELFK........HLRNDLFSYQREVE........LEKFLGCSTQEAAEAVNDLLTSRLQQFENTAL........
WP_012382258  ......FFFDDHFLELFK........HLRNDLFSYQREVE........LEKFLGCSTQEAAEAVNDLLTSRLQQFENTAL........
WP_018956438  ......FFFDDHFLELFK........HLRNDLFSYQREVE........LEKFLGCSTQEAADAVNDLLTSRLQQFENTTL........
WP_018515109  ......FFFDDHFLELFK........HLRNDLFSYQREVE........LEKFLGCSTQEAAEAVNDLLTSRLQQFENTVL........
WP_014158016  ......FFFDDHFLELFK........HLRNDLFSYQREVE........LERFLDCSTQEAADAVNDLLTSRLHQFENTAL........
WP_014143690  ......FFFDDHFLETFK........HLRNDLFSYQRETE........LEKFLHCTTQEAADSVNDLLTSRLQQFENTAL........
ABY50951      ......FFFDDHFLEVFK........HLRNDLFSYQREVE........LERFLGCGTQEAAEVVNDLLTSRVQQFENTAL........
S1            ......FFFDDHFLEVFK........HLRNDLFSYQREVE........LERFLGCTTQEAANAVNDLLTSRLHQFENTAL........
                    ********* :*        *************.*        ** *: * **:**: ***:****::***.*.:
                           462              480    615                      635
Q9X839        ........................LDLASGWLAFGTYGDDYYP........................DYACLLNDVFSYQKEIEYEGE..............................
WP_012999852  ........................LDLSSAWLAWGTYGDDYYP........................DYGCLINDVFSYQKEIEYEGE..............................
WP_010983603  ........................LDLSSRWLAWGTYGDDYYP........................DYGMLINDVFSYQKEIEYEGE..............................
WP_003971162  ........................LDLSSGWLAWGTYGDDWFP........................DYACLMNDLFSYQKEIEYEGE..............................
WP_012382258  ........................LDLSSGWLAWGTYGDDWFP........................DYACLMNDLFSYQKEIEYEGE..............................
WP_018956438  ........................LDLSSGWLAWGTYGDDWFP........................DYACLMNDLFSYQKEIEYEGE..............................
WP_018515109  ........................LDISSAWLAWGTYGDDWFP........................DYACLLNDLFSYQKEIEYEGE..............................
WP_014158016  ........................LDLSSGWLTWGTYGDDWFP........................DYACLMNDLFSYQKEIEYEGE..............................
WP_014143690  ........................LDLASQWLTWGTYGDDYFP........................DYACLLNDVFSYQKEIEFEGE..............................
ABY50951      ........................LELSAEWLTWGTYGDDYYP........................DYACLINDLFSYQKEIEYEGE..............................
S1            ........................LELSAEWLTWGTYGDDYYP........................DYATLLNDLFSYQKEIEVEGE..............................
                                      *:::: **::*******::*                        **. *:**:******** ***
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Fig.1 Amino acid sequence alignment of S1 with its homologs. Shadow boxes indicate Mg^{2+} binding motif of germacradienol/geosmin synthase, "*" indicates exact match, ":" indicates moderate match and "." indicate low match

So far, there is no evidence on regulatory protein that can sense the temperature stress which act as a switch in any *Streptomyces*. To gain better understanding, we characterized the unique sequences into several transcriptional regulatory families. Since this is just a preliminary result, we believe that our progress will uncover a novel temperature sensing mechanism. However, this presumption needs further investigation.

On the other side, we also interested in looking at the differences between temperate and tropical *Streptomyces*. There is no clear proof evidence between the tropical and temperate bacterial genome so far. In temperate zone, the weather range from warm or hot in summer and cold in winter in which bacteria may need extra genes to keep them to adapt with the cold temperature. But different from the tropical zone where the weather is warm year round, they do not need to keep this gene. Since it is unnecessary for keeping of the gene, their size is expected to be small. They supposed to remove all unnecessary functions as keeping or duplication of the genes, they need to use lots of energy. Basically, what we are trying to propose is that the element of sensing temperature is not necessary to be carrying in the genus of *Streptomyces* in tropical country.

5. CONCLUSION

Overall, results presented here are still in the preliminary stage. Genomic analysis and transcriptional regulators characterization provide better understanding on what is happening inside. We are now continuously working deeper into the regulatory mechanism underlying temperature response which may lead to the discovery of the new enzyme or pathway for secondary metabolite production from tropical isolates.

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O3-40

Eutrophication Mapping of Lowland Lakes in Nepal

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Keywords: water quality, Chl-a, eutrophication

ABSTRACT

Eutrophication is a major water quality problem in the lakes of lowland of Nepal, causing turbid water with high algal biomass and offering poor condition loadings of limiting nutrients, such as nitrogen and phosphorus (i.e., cultural eutrophication), to the aquatic ecosystem with dramatic consequences for drinking water sources, fisheries and recreational water bodies. To mitigate these problem this study was conducted to assess the status of eutrophication of Betana Lakes, Rajarani Lakes and Tal Taliya Lakes of Eastern Nepal through remote sensing techniques and comparing the eutrophication indices derived from field data with remote sensed data. For each lake, two sites were sampled and two samples were collected for the measurement of Chlorophyll-a (Chl-a), Phaeophytin, Nitrate, Phosphate, and Ammonia. Chl-a concentration was found to be in the range of 9.6 -38.7 in Betana Lakes, 1.5-38.28 in Raja Rani and 8.9-39.0 in Tal Taliya of lowland Nepal. The remote sensing based Chl-a prediction model was applied where the Landsat visible bands ranging from blue (OLI 2) to green (OLI 4) and near-infrared band (NIR) (OLI 5) were used and applied to obtain the relationship between the sub-surface reflectance and the bio-physical parameters. Highest correlation (R2= 0.9176) was found between in-situ Chl-a concentration and band ratio of the reflectance of Landsat 8 OLI band (B5/B4). Therefore, this band ratio (B5/B4) was considered as the best predictor of Chl-a concentration and further used for developing the prediction model for the eutrophication mapping of the lakes.

1. INTRODUCTION

Inland waters are important natural resources around the world; yet they are threatened by eutrophication due to climate and anthropogenic changes. Eutrophication is a major water quality problem worldwide, causing turbid water with high algal biomass and offering poor condition to the aquatic ecosystem(Portielje and Van der Molen, 1999). Eutrophication occurs naturally over centuries as lakes age and are filled in with sediments(Odermatt et al., 2012).

Chlorophyll-a (chl-a) is a photosynthetic pigment that is found in all green floral components including algae(Wetzel, 2001). However, excessive concentration of Chlorophyll is undesirable as it inculcates eutrophic condition of the lake(Hallegraeff, 2003) and results in increment of phytoplankton standing crop(Boyd and Tucker, 1998). So, Chlorophyll estimation is regarded as an indicator of nutrient status of aquatic body and helpful for monitoring and managing the eutrophic lakes (Carlson, 1977).

Satellite remote sensing is fast and relatively low operational cost process and can be used as a tool to derive spatio-temporal variability in lake water quality (Zilioli and Brivio, 1997).

Landsat imagery has proven to be useful in understanding global trends in landuse change over different timescales. Recently, satellite-derived data are being used to monitor water quality among other things. The 15 m and 30 m resolution of the Landsat 8 Operational Land Imager (OLI) combined with high global data availability, present a unique platform to provide the first and most up-to-date global inventory of the world's lakes and water quality information retrieval at high spatial resolution and positional accuracy using recent Landsat algorithms (Li and Sheng, 2012; Sheng and Li, 2011; Smith, 2005). This study is the first time that a remote sensing technique is being utilized for the measurement of eutrophication and lake management in Nepal.

Objectives

The main objective of this study is to assess the status of eutrophication of various lakes using remote sensing techniques and compare the eutrophication indices derived from field data with remote sensed data. The specific objectives are:

- To determine the effectiveness of spectral reflectance values from the Landsat-8 OLI in respect to measurement of Chl-a and Trophic State Index (TSI) in the Betana Lake of Morang district, Tal Talaiya of Sunsari district and Rajarani Lake of Dhankuta District.

- Compare in-situ Eutrophication index with Remote Sensed Eutrophication index and validate result.
- To analyze the seasonality of the eutrophication at the study sites.

2. METHOD

The Betana Lake in Morang District, TalTalaiya Lake in SunsariDistrict, and Rajarani Lake in Dhankuta District, the lowland lakes of Nepal were selected as the study area to assess the applicability and pilot the application of remote sensing to measure eutrophication levels in Nepal.

Water sampling procedure for in-situ measurement

The in-situ sampling was performed on the prefixed date when Landsat satellite overpasses the Study area. For each lake, two sites were sampled and considered as study points in this study. From each of the sampling sites two samples were collected for the measurement of Chlorophyll-a (Chl-a) as per APHA using the formula;

$$\text{Chl-a} = 11.85(OD664) - 1.54(OD647) - 0.08(OD630)$$

Remote sensing with Google Earth Engine

The Google Earth Engine (GEE), an online environmental data monitoring platform in 30 m spatial and 15 m multispectral resolution incorporates was used for studying inland water bodies of the study areas.

Retrieval of Eutrophication Indices from LANDSAT data

Correlation between OLI brightness values and the water quality parameter by linear regression analysis was used in the study.

3. RESULTS

Table 1 Pearson correlation coefficients (R) between Chlorophyll-a (Chl-a)concentrationand various Landsat OLI bands and band ratios of the study points

Band Combinations	*R*	*Band Combinations*	*R*
B2	0.41	(B3+B5)/2	0.32
B3	0.16	(B2+B5)/2	0.45
B4	0.3	(B4+B3)/2	0.23
B5	0.49	(B4+B2)/2	0.37
B5/B2	-0.54	(B3+B2)/2	0.3
B5/B3	0.009	(B2+B3+B4)/3	0.3
B5/B4	**0.9176**	(B2+B3+B5)/3	0.36
B4/B3	-0.26	(B3+B4+B5)/3	0.31
B4/B2	-0.64	(B2+B4+B5)/3	0.31
B3/B2	-0.71	(B2+B3+B4+B5)/4	0.35
(B4+B5)/2	0.4	**The bold indicates highest correlation**	

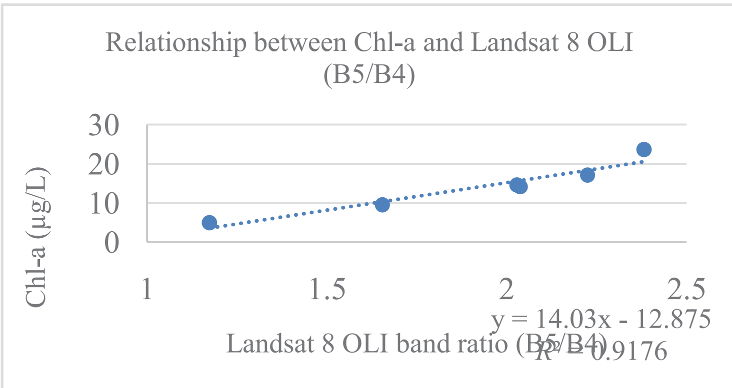

Fig1 Relationship between Chl-a and Landsat 8 OLI band ratio (B5/B4)

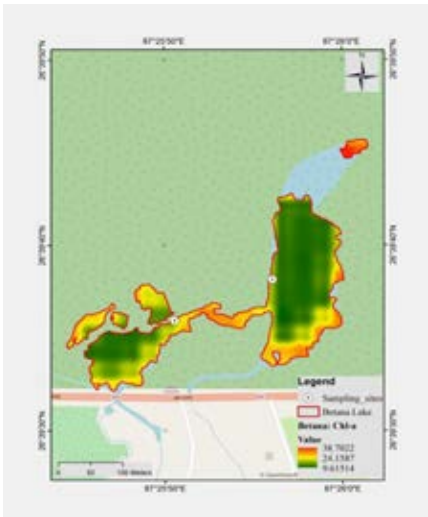

Fig 2 Eutrophication map of Betana Lake

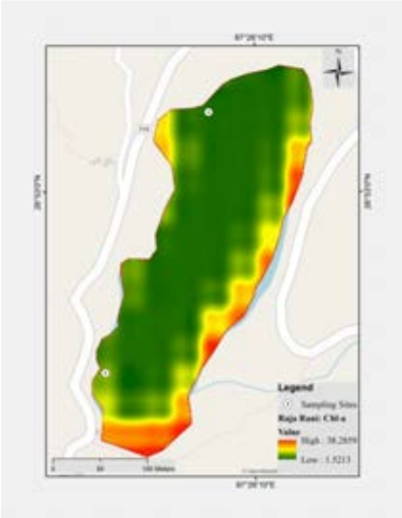

Fig 3Eutrophication map of RajaRani Lake

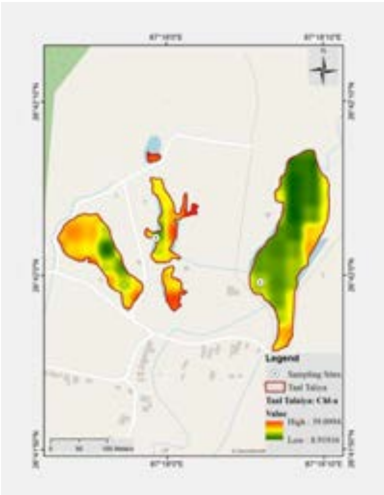

Fig 4 Eutrophication map of TaalTalaiya Lake

4. DISCUSSION

The eutrophication levels at the three lakes studied show that larger water bodies have higher eutrophication levels along the shore line while smaller water bodies from the lake clusters in Betana and TaalTalaiya have larger levels of eutrophication throughout.

5. CONCLUSION

This study was a pilot study to use a remote sensing and GIS techniques in the estimation of eutrophication levels at various lakes in the Eastern Nepal. The results of the prediction model suggest that a similar model is highly suitable (R2= 0.9176) for applications otherwise requiring large inputs of resources and time for the mapping. The surface flow is believed to lead to a higher eutrophication level as it would bring runoff of chemical fertilizers used in the agricultural fields as well as accumulated organic matter in the watershed.

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O3-41

Lakes of the Chernobyl exclusion zone: the effects of long-term radiation exposure on aquatic biota

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Keywords: Chernobyl exclusion zone, lake ecosystems, radioactive contamination, higher aquatic plants, mollusks, fish

ABSTRACT

The effects of chronic irradiation of aquatic biota in lake ecosystems within the Chernobyl exclusion zone during 1998-2017 were studied. It is determined that the rate of chromosomal aberrations in the root meristem tissues of aquatic plants in the most radioactive contaminated lakes on average in 2-3 times, and in cells of the pond snail embryos in 4-6 times exceeding the spontaneous mutagenesis level, inherent to aquatic organisms. Analysis of leukograms of fish peripheral blood showed the decrease of lymphocyte cells, as well as the increase in the number of granulocytic cells (neutrophils and pseudoeosinophils) with increase of radiation dose rate. Along with changes in leukograms an increased level of morphological damages of erythrocytes (structural and proliferation abnormalities) was determined, which is generally for pray fish in 4-12 times and for predatory fish in 7-15 times higher than in fish from reference lakes. Some morphological changes in gills and axial skeleton of fish were determined as well. Analysis of the viability of the seed progeny of the common reed from contaminated lakes at germination in the laboratory showed a reduction in technical germination, germination energy and seed viability with increase of radiation dose rate. At the same time significantly increased the number of abnormalities of seed seedlings in view of necrosis of roots, disturbance of gravitropism, damages of organogenesis and disorder of chlorophyll synthesis were discovered.

1. INTRODUCTION

The Chernobyl NPP (CNPP) accident is a most scale catastrophe in history of nuclear energy both for the amounts of the radioactive matters thrown out in the environment and on the area of contaminated territories. Currently the radioecological situation in the Chernobyl exclusion zone (CEZ) is determined primarily by long-lived radionuclides ^{90}Sr, ^{137}Cs, ^{238}Pu, ^{239}Pu, ^{240}Pu and ^{241}Am. Along with natural decontamination processes in aquatic ecosystems such as physical decay of radionuclides and their hydrological transport outside the CEZ, there is a change of physical and chemical forms of radioactive substances in soils of catchment areas, their transformation and transition in the mobile and bioavailable state, and accumulation by hydrobionts. This is essentially deteriorates the radiation situation in lake ecosystems, which are some kind of “storage system” of radioactive substances in the CEZ, and results in increase of radiation dose to aquatic species that exhibits in a variety of radiation effects at different levels of biological systems.

Thus the main tasks of our researches within the CEZ were: radiation dose rate estimation due to external and internal sources of irradiation for different groups and species of hydrobionts; evaluation of dose-dependent cytogenetic, haematologic, morphometric, productional and parasitological effects due to long-term radiation exposure on aquatic species.

2. METHODS

Our studies were carried out during 1998-2017. The water bodies of research were the flood plain lakes of the Pripyat River within 10-km area around the destroyed unit of the CNPP - Azbuchin Lake, Yanovsky Backwater, Dalyokoye Lake, Glubokoye Lake as well as cooling pond of the CNPP. The results of radiobiological analyses compared to the data received for hydrobionts from the reference lakes with background levels of radioactive contamination.

The concentration of the main dose-forming radionuclides was measured by α-, γ-spectrometry and radiochemical methods. The results were measured in Bq/kg at natural humidity and the mistake of estimated radionuclide concentration fell within 15-25%. The estimation of the radiation dose rate for aquatic species, due to external and internal sources of irradiation was carried with use of ERICA Assessment Tool 1.2.1 [1]. All dose rate calculations performed on the basis of our own data.

The chromosomal aberration rate was measured in embryo cells of the gastropod pond snails by the standard anaphase method [2] and in the apical root meristems of the eight species of higher aquatic plants - by the modified method [3].

Haematological studies of molluscs were carried out in mantle liquid of the pond snails by the analysis of dead cells, young amoebocytes and phagocytic cells quantity [4, 5]. The leukograms and rate of abnormal red cells in peripheral blood of fish were identified by [6, 7].

3. RESULTS

The highest rate of chromosomal aberration in embryo tissue of gastropod snail (*Limnaea stagnalis*) in lakes within the CEZ was found in snails from Glubokoye Lake (up to 27%), located within the dammed territory on the left-bank flood lands of the Pripyat River and characterized with highest density of radioactive contamination (Fig. 1). The rate of chromosomal aberration for snails from the reference lakes was about 1.1-2.0%.

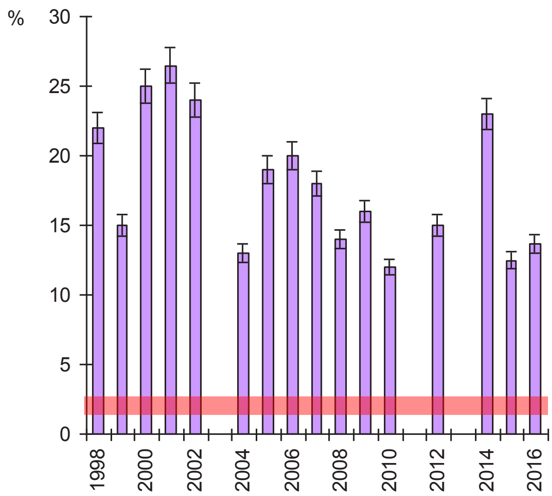

Fig. 1 Dynamics of chromosomal aberration rate in the pond snail (*Lymnaea stagnalis*) embryos in Glubokoye Lake during 1998-2016 (red line – spontaneous level 2.0-2.5%)

The rate of chromosomal aberration in the root meristem tissue of higher aquatic plants from lakes within the left-bank flood plain of the Pripyat River was in 2-3 times higher than spontaneous mutagenesis level.

Analysis of leukograms in peripheral blood of fish showed the decrease of lymphocyte cells, responsible for the implementation of immunological reactions, as well as the increase in the number of granulocytic cells (neutrophils and pseudoeosinophils), responsible for phagocytic function and involved in allergic and autoimmune reactions.

Drawing attention the high rate of red cells aberrations and abnormalities in peripheral blood of fish from the stagnant water bodies within the CEZ, where the absorbed dose rate on the fish organisms due to internal and external sources of irradiation have reached 70-100 μGy h^{-1} that is more on three orders higher in comparison with water bodies with background level of radioactive contamination (Fig. 2).

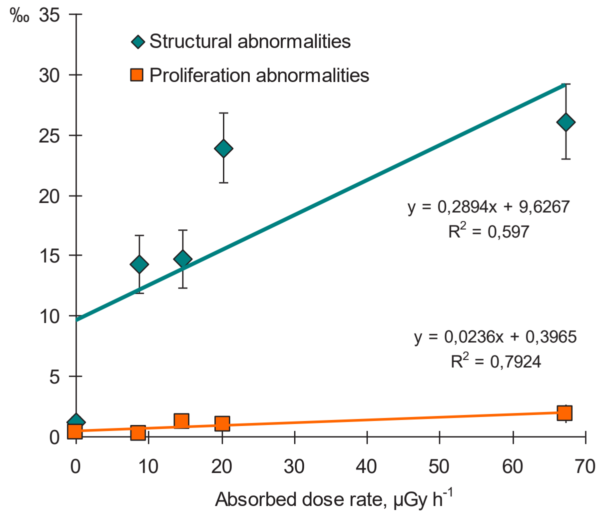

Fig. 2 Dose-dependent rate of red cells abnormalities in the peripheral blood of the common roach (*Rutilus rutilus*) from the CEZ

Our studies in peripheral blood of fish from lakes within the CEZ have shown an increased level of abovementioned morphological damages of erythrocytes, which is generally for pray fish in 4-12 times and for predatory fish in 7-15 times was higher in comparison with individuals from the reference water bodies with background levels of radioactive contamination. The rate of micronuclei in red cells was very low and did not exceed 0.3-0.6‰. For the fish from reference water bodies this type of abnormality are not discovered.

The comparative analysis of morphometric parameters of erythrocytes in peripheral blood of the common rudd showed that corpuscular volume of fish from the reference lake characterised by the homogeneous structure – a high quantity of cells has a similar volume. As to water bodies with medium (CNPP cooling pond) and high levels of radioactive contamination (Glubokoye

Lake), we can observe here an increased tendency of heterogeneity of studied parameters with formation of so-called “double-humped” curve, which is the most expressed for fish from Glubokoye Lake and can testify to the anaemic processes in blood of fish.

During the period of studies we analyzed morphological parameters and the presence of anomalies of egg capsules and egg mass of the pond snails: despiralization or weak spiralization strand with egg capsules; multilane organize of egg capsules in egg mass; location loose of egg capsules; twin egg capsules; polyzygosity of egg capsules; egg capsules without zygotes; zygote outside the egg mass; egg capsules larger or smaller sizes, as well as irregular shape.

Analysis of the viability of the seed progeny of the common reed (*Phragmites australis*) at germination in the laboratory showed that in gradient of absorbed dose rate for parental plants in lakes, there is a reduction in technical germination from 93 to 60%, germination energy - from 91 to 30% and seed viability - from 54 to 38%. At the same time significantly increased the number of abnormalities of seed seedlings: necrosis of roots - from 1.3 to 14.7%; disturbance of gravitropism - from 2.6 to 17.0%; damages of organogenesis - from 4 to 24% and disturbance of chlorophyll synthesis - from 0 to 2%.

4. DISCUSSION

During 1998-2017 a tendency to decrease of chromosomal aberration level in molluscs from all lakes of the exclusion zone was registered. The probabilistic prediction of the chromosomal aberration rate for gastropod snails in lakes of the CEZ have shown that spontaneous mutagenesis level (2.0-2.5 %) can be reach in Azbuchin Lake and Yanovsky Backwater in 2020-s - 2030-s and in lakes Daloykoye and Glubokoye - in 2060-s - 2070-s.

High amount of erythrocytes with structural and proliferation abnormalities in the peripheral blood of fish from lakes with high levels of radioactive contamination allows us to assume that the qualitative indexes of red cells in blood of fish are more sensitive to chronic radiation influence in comparison with the elements of white blood.

A variety of forms of pathological changes in the structure of blood cells, mainly erythrocytes, may indicate low resistance of cytogenetic apparatus of fish in the face of considerable mutagenicity and genotoxicity of environment. In this situation the ionizing radiation causes damage to the lipid structures of biological membranes (e. g. lysosomes) and violation of their barrier functions that ensure compartmentalization in the cell. This leads to disruption of spatial isolation of enzymes to their substrates and release enzymes to further destruction of macromolecules and intracellular structures. As a result, there are changes not only in the cytoskeleton, but also in functioning of all the organelles in the cell.

5. CONCLUSION

Self-purification of closed water bodies within the CEZ is an extremely slow process. Therefore, ecosystems of the majority of lakes, dead channels and backwaters possess high levels of radionuclide contamination of all components.

The established dose-related effects in hydrobionts of lakes within the CEZ indicates a damage of biological systems at subcellular, cellular, tissue, organ, organism and population levels as a result of chronic exposure to low doses of ionizing radiation.

Cumulative radiobiological processes can last for many generations of aquatic biota allowing currently assume the possibility of incomplete realization of the long-term effects of irradiation.

The rate of chromosomal aberrations in cells of aquatic species in many times exceeds the level of spontaneous mutagenesis level to aquatic biota. Increased levels of chromosome damages may be a manifestation of radiation-induced genetic instability, which is one of the main mechanisms for the protection of living organisms from exposure to stressors with subsequent implementation at higher levels of organization of biological systems.

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O3-42

Abundance of Ciliated Protozoans in a Tropical Lake: The Case of Lake Lanao, Philippines

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Keywords: Lake Lanao, tropical lake, trophic status, ciliated protozoans, abundance

ABSTRACT

Lake Lanao in the Southern Philippines is the second largest lake in the country and considered to be one of the ancient lakes of the world. It is best known for its endemic cyprinid fishes but there is a scarce information regarding its trophic status with the use of biological indicators particularly ciliated protozoans. This study was conducted to determine the abundance of ciliates in Lake Lanao across the littoral and pelagic zones of Ditsaan-Ramain, Marawi City, Binidayan, Balindong and Taraka and to evaluate the usefulness of ciliates as indicators of trophic status. Water samples were collected in three replicates using plankton net. The total mean abundance of ciliates in Lake Lanao was 1,071.70 and 1,686.21 (indiv/m^3) for the littoral and pelagic zones respectively; however, the difference was not statistically significantly significant. Both the ciliate mean abundance and the Trophic State Index based on Secchi disk transparency supported an oligotrophic classification of Lake Lanao. Among the measured physico-chemical parameters, only temperature was positively correlated to the mean abundance of ciliates whilst conductivity, pH and dissolved oxygen were negatively correlated.

1. INTRODUCTION

Lake Lanao is the source of power supply in the Mindanao regions but little is known regarding its trophic status with the use of ciliated protozoans as biological indicators. Their abundance increases as the trophic state of freshwater changes from oligotrophic to mesotrophic or to eutrophic condition [1]. Moreover, their grazing activity increases the rate of decomposition and thus their presence and abundance are good indicators of organic pollution [2]. Like other indicator organisms in aquatic environment such as phytoplankton and zooplankton, their diversity becomes low as pollution increases[3]. Ciliated protozoans usually inhabit environments which are low in oxygen concentration. Because of their size that ranges from 20µm to 2mm, their community structure can be studied easily [4]. Lewis (1985) [5] was the first to describe ciliates as part of the protozoan composition of Lake Lanao. However, to our knowledge, there is neither published study on the abundance nor the composition and of ciliated protozoans in Lake Lanao.

This study was conducted to evaluate the abundance of planktonic ciliated protozoans both in the littoral and pelagic zones that can be used as bioindicators of the trophic status of Lake Lanao. Hence, this study provided baseline information on ciliates in the study areas that can be very important in studying the ecological dynamics in freshwater tropical lakes and in giving some insights on the possible Lake Lanao monitoring campaign to maintain a good trophic status and conservation of biological resources.

2. METHOD

Location of the study

This study was conducted in selected areas of Lake Lanao, along the municipalities of Ditsaan-Ramain, Binidayan, Balindong and Taraka, and Marawi City, Lanao del Sur (Fig.1). Ditsaan-Ramain and Marawi City are situated in the Northern and shallower portion of the lake; Ditsaan-Ramain site is near the Ramain River, a lake tributary, while Marawi City is near the lake outlet, the Agus River. Binidayan is the deepest site situated in the southern part while Balindong is in the western part with domestic activities. Taraka is an eastern site locally known as the "basak-area" due to with agricultural activities and near to Taraka River, another main tributary of the lake.

Sampling strategy Abundance and trophic state index

Collection of water samples were done with the use of conical plankton net with mesh size of 53 µm and mouth diameter of 0.48895 m. For the littoral zones, the plankton net was towed obliquely to get an integrated water sample representing the surface and middle parts of the water column. For the pelagic zones, the plankton net was towed vertically at a distance of five meters in each specific sampling site. Only the planktonic ciliates were considered in this study. The actual volume of the water that was sampled was 0.9388 m^3. For every concentrated 10mL of water sample, 1mL of 5% formalin and a drop of acidified Lugol's solution were added after transferring the water samples into the plastic sampling bottles.

Fig. 1 Location of Lake Lanao and sampling sites [27].

Ciliates were counted manually per observation of water samples for fixed samples using the right-hand rule method for microscopy. Abundance (indiv/m^3) of the ciliated protozoans was calculated according to Harris *et al.*, (2000) [6]. Trophic state index (TSI) based on Secchi disk transparency was calculated according to Carlson (1977) [7].

Physico-chemical parameters & statistical analysis

Two physical factors, temperature and Secchi depth and three chemical factors namely pH, dissolved oxygen (DO) and water conductivity were also measured during the sampling using portable devices. All statistical tests utilized Statistical Package for Social Sciences (SPSS), Version 22 namely Shapiro-Wilk test test for normal distribution of the ciliates' mean abundance; Levene's test for equality of variances; t-test for comparing mean abundances; and Pearson test for correlation between ciliates' mean abundances and measured physical and chemical parameters.

3. RESULTS AND DISCUSSION

Abundance of ciliated protozoans

A total of 30 ciliated protozoans observed in this study with a total mean abundance of 2,728.10 (indiv/m^3) (Fig. 2). *Vorticella* sp.3 obtained the highest abundance (567.11 indiv/m^3) while *Spirostumum* sp. obtained the lowest count (0.10 indiv/m^3). As can be seen in Fig. 3, the mean abundance of cilaites in the littoral and pelagic zones vary. Combining all the data of the five sampling sites, the littoral zone has a total mean abundance of 1,071.70 (indiv./m^3) ciliated protozoans while pelagic zone was higher with the value of 1,686.21 (indiv./m^3) but these values are not significantly different using Mann-Whitney Test (Asymp. Sig.: 0.507, α = 0.05). Surprisingly, the abundance of ciliates in pelagic zone of Taraka was significantly higher than its littoral zone. A similar trend

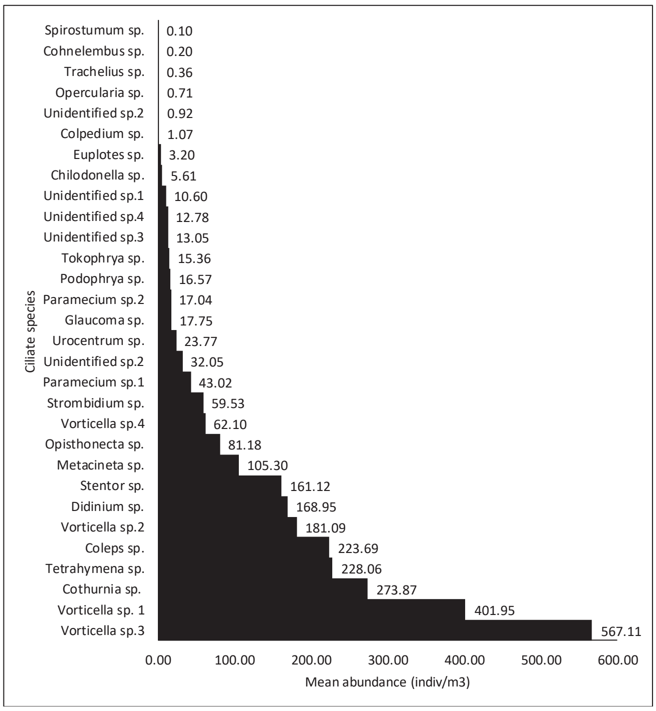

Fig. 2 Total mean abundance of ciliated protozoans

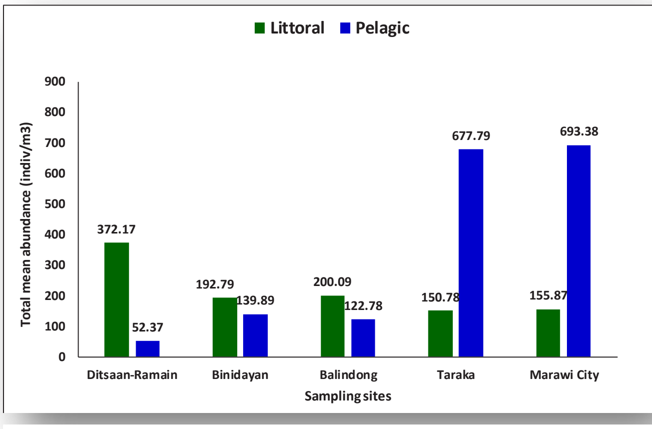

Fig. 3 Mean abundance of ciliated protozoans in the

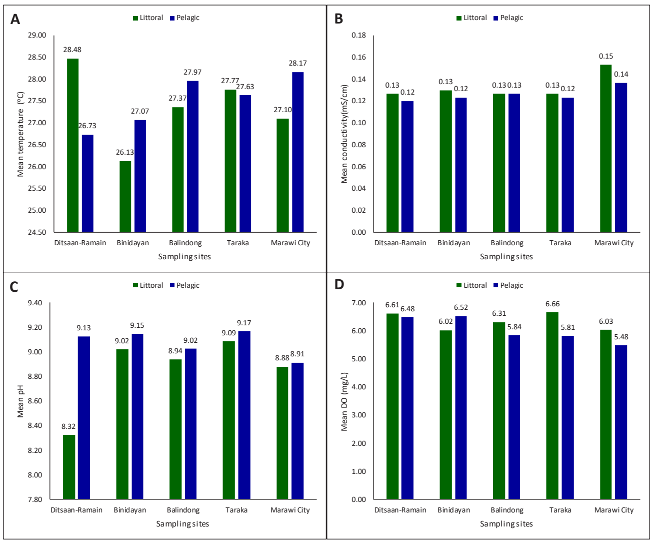

Fig. 4 Measured physico-chemical parameters. A. Mean temperature; B. Mean conductivity; C. Mean pH; D. Mean dissolved oxygen (DO)

was also observed in the Marawi sampling site. Based on ocular observation, the Taraka site has relatively turbid water both in the littoral and pelagic zones as compared to other sites. Since the mouth of Taraka river is close to the sampling site, this might account for the turbidity reaching the pelagic zone of which ciliated protozoans might be carried by the water current along with some debris and organic matters. In the case of Marawi, this could be attributed to the domestic activities such as bathing and washing of the inhabitants near the sampling site. In fact during sampling, direct discharge of soaps and detergents in the sampling area was observed.

Physico-chemical parameters

The physico-characteristics of the sampling sites are shown in Fig 4. The temperature of the five sites ranged from 26.13°C to 28.48°C (Fig 4A) and showed a positive correlation of 0.244 with ciliate abundance by Pearson test. This is expected though since temperature increases as the rate of metabolic reaction increases [7]. For the conductivity, the different sampling locations have relatively uniform values of 0.12 – 0.13 mS/cm (Fig. 4B) having negative correlation (-0.044) with abundance by Pearson's. This result is in contrast to the study of Yasindi

& Taylor (2006), in which conductivity and trophic status were the most important environmental variables influencing the distribution of ciliate species in East African lakes [8]. For pH, the values ranged from 8.32 to 9.17 (Fig. 4C) having a -0.175 Pearson correlation with ciliate abundance. Lake Lanao is expected to have high pH based on the study by Beaver and Chrisman (1989) [9] on subtropical lakes in which abundance and biomass of total ciliates generally decreased with increased acidity.

For DO, the highest value recorded was 6.6g mg/L in the littoral zone of Taraka while the lowest was the pelagic zone of the same site with the value of 5.81 (Fig.4-D). The Pearson correlation analysis on DO and ciliate abundance revealed a negative (-0.241) value in which the sampling sites with high abundance had low levels of DO. Across all sites, the pelagic zone of Taraka marked the lowest record of DO but it also hit the highest record of ciliate abundance. This result was consistent with the study of Dias *et al.*, (2008) [10] in the urban stream of southeast Brazil in which low DO were recorded in sampling sites with high ciliates abundance and these sites were receiving high sewage loads indicative of organic pollution. Taraka also had a lower DO and it was corroborated by high ciliate abundance. This was probably due to the agricultural activity near the site and its nearness to the lake tributary Taraka River, which was evidenced by the turbid run-off waters.

Moreover, *Vorticella* spp. were accountable for the alarmingly high abundance of ciliates in the pelagic zone of Taraka. This result is not surprising since *Vorticella* ciliates are strongly related to organic pollution. As early as 1952 *Vorticella* spp. were already listed as typical indicators of organic pollution from moderate to heavily polluted condition. Higher counts of the same genus were also observed at littoral zone of Binidayan. Though this sampling site was deepest part of the Lake but it was surrounded with households. In addition, in the littoral zone of Ditsaan-Ramain, high abundance of *Vorticella* sp. was also accounted. Ditsaan-Ramain site was shallower and received waters from one of the main tributaries of Lake Lanao – the Ramain River. The site was exploited for fishing and domestic uses. Probably during sampling, these sites mentioned most likely received higher loads of domestic waste as indicated by higher abundance of *Vorticella* spp.

Trophic State Index (TSI) and Ciliate Abundance

Trophic status of the lake can be indicated in two ways, namely TSI based on Secchi depths following the work of Carlson (1977) [7] and the standards set by Beaver and Crisman (1989) [9] based on ciliate abundance. Littoral zones of Ditsaan-Ramain and Balindong had no Secchi depth values because the water levels were shallow and clear enough to see the Secchi disk at the bottom. The sampling sites of the pelagic zone had Secchi depth values of 4.33m – 5.85m (data not shown). Calculating these values to TSI yielded a range from 34.51 to 47.83 which are interpreted as oligotrophic category [12]. This is also confirmed by comparing the abundance of ciliated protozoans in this study against the standards set by Beaver and Crisman (1989) [9], in which Lake Lanao is considered as ultraoligotrophic, i.e. lake water in the sampling sites is still pristine.

5. CONCLUSION

Based on the total mean abundance of ciliates and Secchi depths, Lake Lanao is classified as oligotrophic lake. Ciliated protozoans in the lake did not significantly vary in the littoral and pelagic zones during the time of sampling. Furthermore, for the physico-chemical parameters, only temperature was positively correlated to the mean abundance of ciliates whilst conductivity, pH and dissolved oxygen were negatively correlated. Thus, it is highly recommended to obtain comprehensive data on ciliated protozoans and other biological indicators to generate an overall picture of the dynamics of the biological community in Lake Lanao ecosystem. Public policy and ordinance on bio-monitoring and assessment of Lake Lanao by the concern local government units is also needed. This is to preserve the Lake Lanao and prevent any further degradation that is influenced by anthropogenic activities.

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